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## General Methods in Botanical Microtechnique.

### I.

In the following account, I have attempted to give only such methods and reagents as are required to carry on successful work and which I have found to be the best in my own investigations. All details are carefully stated, so that a beginner should be able, with little outside help, to carry the operations through successfully. The methods employed in preparing plant tissues must be considerably different from those used in zoölogy, since we usually have to deal with a thick cellulose wall and a very delicate protoplasm in which are usually contained large vacuoles filled with cell sap, besides numerous plastids and food contents, all of which tend to make it difficult to preserve and study the finer details of structure in plant cells and tissues.

The objects taken for a trial study may be some root tips of the common onion (*Allium Cepa*), or pieces of the young ovaries of some species of lily, as *Lilium Longiflorum* or *L. Philadelphicum*. The root tips may be grown by placing an onion in a flower pot with moist sawdust, and keeping it for a few days where the roots will grow rapidly. The tips should be cut from one-half to three-fourths of a centimeter in length. The lily ovaries may be taken at various stages before and after the flowers open, and cut into transverse pieces from one-fourth to three-fourths of a centimeter long.

### 1.—KILLING AND FIXING.

The first thing to do in beginning to prepare any plant tissue for permanent mounting is to kill and fix it in such a way that it will preserve the minute structures as near the living condition as possible. A sharp knife or scalpel should always be used, and great care taken so as not to bruise or injure any of the cells.

*Killing Fluid.*—The killing fluid is made up as follows:

1. Chromic Acid	-	-	-	-	0.8 cc.
2. Glacial Acetic Acid	-	-	-	-	0.5 cc.
3. Water	-	-	-	-	99.0 cc.

Have the killing fluid in a 4 oz. (120 cc.) bottle with a common cork. Sixty cubic centimeters (2 oz.) of the fluid will be enough to kill one or two dozen objects the size of the root tips. The material must be perfectly fresh and put into the killing

fluid as soon as cut. They will usually sink to the bottom after a short time, especially if they are shaken a little from time to time. If much trouble is experienced in having the objects float on the surface of the killing fluid, they may first be immersed for a very brief moment in 95 per cent. alcohol, and immediately after dropped into the killing fluid. This will cause them to sink. The objects must be kept in the killing fluid from twelve to twenty-four hours. The onion root tips should be left at least twelve hours; for larger objects, a proportionately longer time.

## 2.—WASHING.

After the tissues have been thoroughly killed and fixed, the next thing necessary is to wash out the acid. This may be done by pouring off the acid and filling the bottle with water, and changing from time to time. They should be washed in this way from one to four hours, depending on how often the water is changed. A better way, however, is to set up and use the apparatus described as a convenient washing apparatus by the writer.\* The water used for washing should be rather pure. If this is not the case, distilled water had better be used.

## 3.—DEHYDRATING AND HARDENING.

The next step is to remove all water from the tissues, and this must be done very gradually or the tissues will shrink and the protoplasmic contents of the cells will be distorted so that the preparations will be worthless. To remove the water successive grades of alcohol are used. During this process the objects may still be kept in the same bottle. The amount of each grade of alcohol should be sufficient to cover the objects well. The various grades of alcohol should be made up and kept in a special set of bottles. It is best not to use the alcohol more than once for this process. Carry them through in the following order :

- |    |                      |   |   |        |        |
|----|----------------------|---|---|--------|--------|
| 1. | 10 per cent. Alcohol | - | - | 4      | hours. |
| 2. | 25 per cent. "       | - | - | 4 to 8 | "      |
| 3. | 35 per cent. "       | - | - | 4 to 8 | "      |
| 4. | 50 per cent. "       | - | - | 4 to 8 | "      |
| 5. | 70 per cent. "       | - | - | 48     | "      |

The objects should be hardened in the 70 per cent. alcohol at least two days, and a longer period is generally better. They may be kept in 70 per cent. alcohol for several months without injury.

- |    |                      |   |   |        |        |
|----|----------------------|---|---|--------|--------|
| 6. | 85 per cent. Alcohol | - | - | 12     | hours. |
| 7. | 95 per cent. "       | - | - | 4 to 8 | "      |
| 8. | 100 per cent. "      | - | - | 4 to 8 | "      |

As a general rule, it is convenient to make three changes a day, morning, noon, and night, except the 70 per cent.

## 4.—CLEARING.

The objects must now be put into some fluid which will dissolve paraffin. The best reagent for this purpose is chloroform.

1. Add one-third chloroform to the absolute alcohol. Let stand from four to eight hours.

\* Description will appear in February JOURNAL.

2. Add enough chloroform to make a two-thirds solution, and let it remain from four to eight hours.
3. Transfer to pure chloroform and leave from six to twelve hours.

#### 5.—IMBEDDING IN PARAFFIN.

The objects are now ready for the paraffin. This should be of good quality, with the melting point at 49 degrees or 50 degrees C. The paraffin must be added gradually, in the following manner: add small pieces of cold paraffin to the chloroform in which the objects are, sufficient to form a cold saturated solution. After the cold chloroform has taken up all the paraffin possible, say after about six or eight hours, the objects must be gradually brought into the hot water oven. This may be of various designs and sizes. A square oven with a side door is very convenient and cheap. The oven should be kept at a uniform temperature of about 52 degrees C. The bottle may first be placed on top of the oven, and then inside with the door a little open, and finally with the door closed. When warmed up to the temperature of the oven, melted paraffin, kept in a suitable dish in the oven, may be added from time to time, at intervals of two or three hours. At the same time some of the mixture of chloroform and paraffin is poured off until the objects are in pure melted paraffin, with all traces of chloroform removed. The objects should stay in the oven at least a day, and several days will do no harm if the temperature is uniform. I usually take two days for the operation. One day, however, is usually long enough unless the objects are very large and difficult to penetrate.

#### 6.—MAKING THE CAKE.

The final imbedding can be easily done in the following manner: use a Petri dish of proper size, 80, 120, or 150 mm. in diameter, depending on the amount of material to be imbedded; or the paraffin imbedding dish described by the writer in Vol. 1, page 11, of this journal. Before imbedding, apply a very thin coat of a 50 per cent. aqueous solution of glycerine to the parts of the dish with which the paraffin will come in contact, and pour in a suitable amount of melted paraffin to make the cake. The objects being in the bottle with the cork, turn the bottle upside down and allow the objects to settle on the cork. Then remove the cork and let the paraffin in the bottle, with the objects, fall into the dish. The objects may be arranged in the paraffin with hot needles. Put the dish quickly into cold water, but do not let the water flow into the dish until the paraffin is hard enough to bear the weight of the water without being distorted. The paraffin cake must be cooled very rapidly, and this is usually done best in cold flowing water. After the cake is thoroughly hardened it is carefully removed from the dish and laid aside until used. When the objects are once properly imbedded they can be preserved for an indefinite period if kept in a cool place. The bottle in which the objects were kept while passing through the paraffin may be used for the same purpose for subsequent imbeddings. After the objects are in pure chloroform they can be poured into this bottle, which will already have some paraffin adhering to its walls.

JOHN H. SCHIAFFNER.

## Notes on Preparing Foraminiferal Material for Study.

In the unconsolidated deposits of cretaceous and more recent age, foraminifera may be looked for in the marls, bryozoan deposits, calcareous sands, or fine sands and clays. The coarse sands contain few or none, while the finer materials—the products of slower sedimentation—are often quite rich. The filling within the shells of bivalves is often much richer than the matrix around these fossils.

The material to be examined should be thoroughly dried and all masses or lumps broken up. If the material—as for example, the bryozoan sands—contains some of the larger or longer forms, it may be searched at once. If, however, only the more rotund and smaller forms are present, any coarse shell fragments or other large-sized material may be separated by sieving the material, using a mesh large enough to let the foraminifera pass through. If this sieved material contains a large proportion of exceedingly fine clay particles, these may be gotten rid of in a similar way—by using a bolting cloth sieve that will let the clay particles pass through but retain the material of about the size of the foraminifera—say all larger than 0.2 to 0.4 mm.

This mechanical separation may, in material of some textures, be carried a step further by placing the material in a paper and gently shaking it in such a way as to keep it in a rather compact mass but vigorously enough to let the particles move freely among each other. There will be a tendency for the foraminifera and other of the lighter constituents to work their way to the surface and concentrate there, as it were.

A pocket magnifier with a power of say six to twelve diameters, and as large and flat a field as possible, is necessary, aided by a pair of sharp eyes. After finding a specimen it may be readily picked out by touching it with the sharp point of a fine, well moistened camel's-hair brush, or with the moistened end of a rather blunt needle, or of a sharpened match or toothpick.

For studying, one must be able to turn the unmounted specimen into any position while under the microscope. This may be readily accomplished by holding it slightly entangled in the hairs of a dry camel's-hair brush. For study in certain positions, it may of course be simply laid on a glass slide. The external surface may be studied to best advantage when perfectly dry. Permanent mounts show the external characters well when strewn over a black surface, e. g., asphalt. To protect from accidents the mount should be surrounded by a gutta percha ring. When mounted in balsam with a cover-glass, the external character is seen with difficulty, while more or less of the internal structure, according to the species studied, becomes visible.

To make sections showing the internal structure, the smaller specimens may be imbedded in hard balsam and ground down very carefully until the median, or desired, section is reached. It may then be mounted section side up, or this side may be cemented on a slide and the opposite side ground down also. For larger individuals the grinding may be easily accomplished by holding the specimen under the ball of the finger and rubbing gently on a piece of smooth ground



glass. If the siliceous or calcareous nature of the test cannot be determined by the eye, resort may be had to chemical means.

L. C. GLENN.

Johns Hopkins Geological Laboratory, Jan. 20, 1899.

## A Rapid Method of Paraffin Imbedding.

This subject has been treated of in numerous magazine articles, appearing from time to time in different journals, and by different writers. These methods, as described by the various authors, take from thirteen to twenty hours in their consummation.

The method, to be described, is actually rapid, and has the added virtue of being extremely useful for diagnostic purposes; and the specimens, so obtained, are permanent.

The method, in detail, is as follows: a piece of fresh tissue, the thickness of a thin or medium microscopical glass slide, is suspended in absolute alcohol, from two, to two and three-fourths hours; it is then placed in benzol-cedar-wood oil mixture until semi-transparent, and no whitish areas appearing in any part of it (usually from ten to thirty minutes), the tissue is now put into melted paraffin, heated to not less than 47 degrees C. or more than 50 degrees C. This paraffin is a mixture of one part hard paraffin (50 degrees C. melting point) and two parts soft paraffin (40 degrees C. melting point). The bath, in paraffin, should be prolonged until the tissue is opaque, as it was at the end of the alcohol bath, —if it is at all translucent it must be returned to the paraffin bath until opaque. The specimen, at this point, should be carefully watched, as some tissues will begin to shrink as soon as infiltration is complete, and should be removed and imbedded at once. Other tissues, that do not show this tendency to shrinkage, may be left in the paraffin bath indefinitely, without detriment.

This bath should require from five to thirty minutes.

The specimen is now imbedded in melted paraffin, of a mixture of two parts of hard and one part of soft, and allowed to cool slowly until semi-solid, when it should be rapidly cooled in ice water.

Sections are cut; affixed to slide with Mayer's albumen mixture; passed through benzine and 90 per cent. alcohol; stained first with dilute aqueous solution of China blue or Bleu de Lyon, rinsed in water, and then brought into safranin solution, for a few seconds; washed with absolute alcohol, until the blue becomes prominent; cleared with clove oil and mounted in Canada balsam. The safranin solution is made by adding one part 40 per cent. formalin to four parts of saturated aqueous solution of safranin.

If a more selective stain is desired, for the demonstration of karyokinetic nuclei the section should first be stained with safranin; washed lightly in 70 per cent. alcohol, containing .5 per cent. picric acid, and finally in absolute alcohol. The tissue, so treated, may be of any length or width, but must not be thicker than mentioned above. The fixation and hardening is accomplished by the use of a four-ounce bottle, the bottom of which is covered to about an inch in depth with burnt copper sulphate and then filled to the shoulder with absolute alcohol;



this amount can be used for twelve or fifteen specimens, or used until the  $\text{Cu SO}_4$  becomes blue, when it must be changed. One end of a thread is attached to the stopper and the other end supports a bent pin, the thread being of such length as to permit of the pin resting just below the surface of the alcohol. The tissue is hung on this pin, and the whole dropped into the alcohol—the bottle being tightly corked.

By preference the benzol-cedarwood oil mixture should consist of one and one-half parts of *thick* cedarwood oil and two and one-half parts of pure benzol.

If thin cedar oil is used the mixture then should consist of equal parts. The thin oil does not give as good results, however.

I have used this method for four years, and in my hands it has been much more satisfactory than the freezing methods. The entire process can be carried through in from three and one-half to four hours.

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## Methods of Mounting Small Coleoptera and Other Small Insects.

In response to the request appearing in the November number of this journal, for a method of mounting small Coleoptera and parts of insects, I will give the methods in use in the laboratory of the Ohio State University.

The method giving best results for general work is as follows: drop specimens in absolute alcohol and leave for an hour or more to dehydrate, then transfer to xylol or other clearing agent for a few minutes, from which they are taken and mounted in balsam. If it is desired to make opaque objects transparent, they should be boiled for a moment in caustic potash and then treated as above described.

If it is desired to clear and mount small and fragile specimens with little handling, this may be accomplished by dropping them in water-free carbolic acid to kill, dehydrate, and clear. After remaining in this an hour or more they are mounted in balsam.

The smaller beetles, after being chloroformed, are often mounted directly in balsam. They are cloudy for a time when mounted in this manner, but in the course of four or five weeks they become clear, so that this simple method may be used to advantage with small insects and their parts if they may be given time to clear up perfectly before being studied.

In working with scale insects (Coccidæ) the insects are first picked out of the scales and placed upon a slide where it is desired to mount them. A few drops of a five per cent. solution of caustic potash are applied, and the specimens boiled in this for two or three minutes by holding the slide over a bunsen burner. The specimens are then worked two or three times by being covered with a few drops of absolute alcohol, after which they are cleared and balsam applied.

E. L. FULLMER.

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## The Preparation of Nucleated Blood in Bulk.

Chloroform the animal, open the thorax, puncture the aorta, and allow the blood to flow directly into a small glass jar, with ground stopper, containing a one per cent. aqueous solution of osmic acid.

The solution should be largely in excess of the amount of blood, at least fifty times as great. The vessel is now closed and set aside in the dark for several hours. The blood cells will in this time have become thoroughly fixed and hardened and have settled in a thin layer at the bottom. Decant the supernatant fluid and add distilled water, gently agitating the vessel until the blood is thoroughly mixed with the water. Again decant after sedimentation has taken place, or filter rapidly through very thin filter paper and wash off the filtrate in a small quantity of distilled water. Next, add Bhomer's hæmatoxylin, diluted with one-half its bulk of distilled water. Use no more of this mixture than enough to promote quick and thorough admixture with the water containing the blood.

After a few moments' staining, filter as before, wash the filtrate from the paper by agitating in a dish of distilled water.

A considerable quantity of water is now added, and the vessel set aside for at least an hour, in which time the nuclei of the cells will be well differentiated.

Dehydration is now accomplished by running the blood through various strengths of alcohol, beginning with seventy per cent. and ending with absolute; filtration or decantation being practiced each step. Care must be taken not to use too small quantity of alcohol, or the cells will not be well dehydrated. Clear in carbol-xylol (carbolic acid one part, xylol two parts), allow the blood to settle in a large test tube or conical glass, draw off as much as possible of the fluid with a large bulb pipette, and add thin xylol balsam.

The blood will keep indefinitely, and it is only necessary to put a small drop of the balsam upon a slide and cover with a cover glass to obtain a beautiful and permanent mount.

It is probable that a two per cent. aqueous solution of formalin will answer as well as the osmic acid.\*

T. E. OERTEL.

University of Georgia.

\*Kizer, E. I. Formalin as a Reagent for Blood Work. Jour. App. Micros. 1: 189, 1898.

## Some Improvements in Laboratory Tables.

Up to the présent year, oak tables have been used in this laboratory. They have not proved entirely satisfactory, for two reasons: first, when cultures are spilled, it is impossible, on account of the absorbing qualities of the wood, to thoroughly disinfect; secondly, drops of stain, which are not easily removed, soon give the tables a very untidy appearance. Also, a few slate tops have been used by way of experiment. While slate is better, in some respects, than oak, yet it is not desirable, for it continually soils the hands and clothes of the worker. At the opening of the present college year, the laboratory was fitted out with glass top tables. The table is covered with corrugated rubber matting, which is of

the same size as the top of the table, and on this pad is placed a piece of plate glass, which projects about one-half inch beyond each edge of the table. The rubber matting prevents the glass from slipping, insures, to a certain extent, against breakage, offers a good background, and takes up much of the vibration. The glass removes the difficulties met with in the use of either wood or slate; it can be thoroughly disinfected, is easily cleaned, and is not unpleasant for the one at work. It is found convenient to place colony counters and whatever measurements or formulas are frequently used in the space between the glass and the rubber matting; here they are always ready for use and, at the same time, are never in the way. Furthermore, the glass adds much to the general appearance of the laboratory. During the three months which it has been tried, this top has proved satisfactory.

In addition to the rubber matting already referred to, another means of reducing the vibration is found in the use of rubber corks placed in the bottom of the table legs. A hole one-half inch in diameter is bored in the center of each leg, and in this hole is inserted a rubber cork of such size that it extends about one-eighth of an inch below the end of the table leg. While these do not eliminate all the vibration, yet they are found to be quite efficient.

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## The Management of a Botanical Laboratory.

Twenty-five years of experience in the management of botanical laboratories may be my excuse for writing upon this topic, in the hope that some of the suggestions may be of service to the younger men who have to face the problem of building up a laboratory with limited means.

*The Outfit.*—It is scarcely necessary to say, that the room assigned to the botanical laboratory should be well lighted, and that north windows are preferable; that there should be water-taps, sink, draining board; that there should be gas laid on, and with facilities for lighting and for free hose connections. There should be solid tables of convenient form (I like V-shaped tables with heavy oak tops, placed in front of each window) of the proper height (73 centimeters), and on these should be gas-pipes with facilities for lighting and hose connections. Chairs or stools of proper height (kitchen chairs are cheap and good, not as good perhaps as the stools which may be adjusted to different heights), blackboards, and the necessary shelving for material scarcely need comment. Individual lockers are quite necessary and may be made at small cost, although the "Cornell Laboratory Locker" (see this JOURNAL, February 1898) is much more useful. The microscopes should be of the low "continental" form, and supplied with at least two objectives (one-half and one-sixth inch) and two oculars, one of which should contain an ocular-micrometer, and must be so solidly constructed as to endure a good deal of wear and tear. A double nosepiece is desirable, but not indispensable. Rack and pinion for coarse adjustment is not desirable for use in the earlier part of the student's work, since it seems impossible thus far for makers to

construct cheap and yet durable rack and pinion attachments; it is much better to have good sliding tube stands than shaky or hard-working rack and pinion instruments. A good fine adjustment is essential, and here again there is much to be desired in most low-priced stands. Makers should seek simplicity and durability here also, since a defective or worn-out fine adjustment is exasperating.

On the tables there should be conveniently placed bottles of a few reagents and stains, those less commonly used being kept on a shelf at the side of the room. The bottles should be broad-based and as low as possible. The stopper should be of glass (with some exceptions, as in case of potassic-hydrate bottles), but very good work may be done where perforated cork stoppers (each containing a small glass tube) are used.

A good razor, scalpel, forceps, dropping tube, several dissecting needles, one or two sable brushes, a supply of glass slips and cover glasses (I prefer 19 mm. circles) several slide boxes ("Pillsbury" boxes are inexpensive, but troublesome), a high-grade drawing pencil (Faber HHHHHH, or its equivalent), an eraser, and a laboratory note-book containing loose perforated sheets of drawing paper (I prefer sheets about 19x24 centimeters) should be purchased by each student for his own use. These will cost from \$3.50 to \$4.00.

The foregoing outfit, which will enable the student to do what we may call elementary laboratory work, must be enlarged if more advanced work is contemplated, by a supply of imbedding material, paraffin baths, paraffin ovens, bottles, tube vials, bell-jars, beakers, shallow dishes, and other glassware, one or more microtomes (the latter so made as to "ribbon" the sections), and a much larger assortment of reagents and stains (of the latter I prefer those made by Gruebler of Leipzig).

*Management.*—With the foregoing summary statement of the outfit of a botanical laboratory, what shall be its general management? Here I may be permitted to base my suggestions upon an experience with a large number of students of all grades, from those with a minimum of previous training, to graduate students with from four to six or more years of laboratory training.

The laboratory should be an "open" room, to which those who are working in it should have free access. For many years it has been my practice to keep my laboratory door unlocked from 8 A. M. to 6 P. M. for six days every week, and to endeavor to make every student feel that he has a right to work as often as he wishes. Of course each man has his regular hours when he is expected to be at work, just as he is expected to regularly attend lectures, but over and above this I have found that many a student enjoys, seldom abuses, the privilege of dropping in for an extra hour or two of work when the spirit of botany moves him so to do. A laboratory assistant is present at all times during the day, so that the student coming in at odd times always finds some one who can render any required assistance. As a result of this practice it is never necessary to "keep hours" upon students, for the spirit of work so increases with the freedom allowed that such a thing as shirking is practically unknown. On holidays, when the laboratories are supposed to suspend all work, the assistants are always met with requests from students for permission to work.

That each student should have his own place in the laboratory, and use but



one microscope, is so obvious as to need no emphasis here. In many of the rapidly growing institutions, it is impossible to avoid "doubling up," a particular microscope and table being used by two students—at different times, of course. When the numbers are large (as in general botany, where the number may be from fifty to one hundred or more) it is necessary to keep the work fairly together. For this purpose the material to be used during a period of a week or so is kept in suitable dishes or jars, on a centrally placed table, and the sequence of the successive "studies" ("observations") is indicated on the blackboard. I have never liked the plan of outlining the steps in each study, but prefer to let each student work out his problem for himself, aided of course by judicious suggestions from the laboratory assistant. Each student is expected to do all the work connected with the particular "study" in hand, and the assistant never does any part of the work for the student. Doubtless less ground is covered by this practice, but the student has learned every step of the work, and is able to repeat it in his own laboratory when he turns teacher after graduation. I have seen laboratories in which the sections were cut by the assistant, who distributed them to the students ready for mounting, and I have known the assistant to accommodately mount these nicely cut sections, leaving nothing but the drawing for the student.

Order in the laboratory? Yes, there must be order, but it should be the order which exists in a home, or a shop, or the salesroom of a business house. I like to walk through my laboratory and hear the low talking of the interested workers; it is much more pleasant and conducive to good work than that class-room-like silence which some advocate. The laboratory is a workshop in which each man has a personal interest in the workroom, and like workers they work and chat easily about what they are doing.

In connection with the mechanical work in the laboratory, it must not be overlooked that records must be made, and so one of the things to be insisted upon is the making of intelligent notes and drawings. Meaningless notes are almost as bad as none at all. So also drawings, even if excellently executed as works of art, have no reason for existence unless they have a significance. The mere making of a drawing has in itself little value, but it must be discriminately made. It is the part of the teacher in charge or his assistant to help the student to acquire the ability to distinguish what is worth recording in words and lines from that which is meaningless and therefore not worth recording.

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The University of Nebraska.

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When a carmine stain is to be used the results may be obtained quicker by heating the stain. Place the watch glass containing the stain on a wire netting over the opening of a water bath. As the water boils the heat of the steam will cause the stain to penetrate more rapidly, with the result that the details of the specimen are brought out more sharply. These results may be obtained only with tissue which has been previously hardened. Those hardened in a solution of chromate of potash to which a few drops of chromic acid have been added give the best results.



## LABORATORY METHODS IN BACTERIOLOGY.

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### V.—Preparation of Culture Media.

A number of nutrient media are employed in the laboratory for the purpose of cultivating bacteria. Three of these are especially important, inasmuch as they are used almost daily. They are gelatin, bouillon, and agar. These standard culture media may be readily modified for special purposes by the addition of glucose, glycerin, or litmus. Although the details for the preparation of these common culture media are not lacking, either in text-books or in current journals, it may not be amiss to describe the methods as followed in the Hygienic Laboratory of the University of Michigan.

#### NUTRIENT GELATIN.

Place five hundred grams of chopped lean beef in a beaker or flask, or better in an enamelled jar such as is shown in Fig. 1.

Now add 1000 cc. of tap-water and stir thoroughly. Immerse the jar in a water-bath and warm gently till the temperature of the meat suspension reaches 55 to 60 degrees C. Maintain this temperature for three-quarters to one hour, stirring frequently. The soluble constituents are thus brought into solution. Special care should be taken not to allow the temperature to rise above 60 degrees, inasmuch as the albuminous substances would then coagulate. By keeping these in solution at this stage, they will subsequently assist in clarifying the final product, and hence the addition of the white of an egg will be unnecessary.

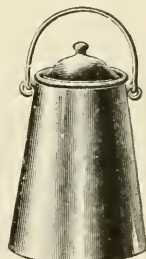


Fig. 1.

When the digestion is completed strain the liquid through muslin and thoroughly squeeze the residue. The filtrate is dark red in appearance and should measure 1000 cc. To this amount of the filtrate returned to the jar, add 100 grams of gelatin, 10 grams of Witte's pepton, 5 grams of common salt, and warm at 60 degrees in the water-bath with constant stirring, till the gelatin has completely dissolved. The next step is to render the liquid slightly but distinctly alkaline.

The neutralization is usually accomplished by the cautious addition of a saturated solution of sodium carbonate. After each addition of one to two cubic centimeters of alkali, the liquid is well stirred and a drop taken out by means of a glass rod, and touched to a blue litmus paper. If the reaction is acid this paper will turn red. The addition of alkali is continued until the paper remains blue and a red one turns slightly blue.

In the hands of the beginner this procedure not infrequently fails because of the difficulty of judging of the end reaction. Moreover, even the practiced eye cannot establish the same degree of alkalinity in two separate preparations. For these reasons some workers prefer to titrate the solution with an alkali of known strength, using phenol-phthalein as an indicator. The latter is a most delicate

indicator when mere aqueous solutions of acid and alkali are to be tested. In the presence of organic matter, ammonium salts, and carbonic acid, it ceases to be a sharp indicator. Moreover, the neutral point as obtained with phenolphthalein does not correspond with the neutral point obtained with litmus. Consequently it has been found necessary to deduct twenty or twenty-five cubic centimeters, from the total amount of alkali necessary to neutralize a liter of the medium. The amount thus subtracted is so arbitrary that the resulting reaction cannot be duplicated, except approximately, in another batch of the same or of other media. Inasmuch as litmus has always been employed for neutralizing purposes, it is well to adhere to this indicator, especially if the object sought for can be attained with ease and exactness.

The following method, as devised by the author, determines, with reference to litmus, the neutral point of any medium, whether gelatin, bouillon, or agar,\* to a degree of exactitude that leaves nothing to be desired. The beginner, with no previous knowledge of quantitative analysis, can impart any desired degree of alkalinity (or acidity) to a given medium.

Two solutions of sodium hydrate are necessary: (1) One that will contain forty grams of this base in one liter of water. This is known as normal sodium hydrate. To prepare this solution so that it will have exactly the strength, requires some experience in chemical work. For practical purposes it is sufficient to dissolve forty grams of sodium hydrate in distilled water and to dilute this solution to one liter. This will give an approximately normal solution. (2) One that will contain four grams of the base in one liter of water. This is known as a decinormal solution. It is prepared by taking 100 cc. of the normal solution and diluting it to one liter. It is evident that this solution has one-tenth of the strength of the former. In other words, 10 cc. of the No. 2 solution has the same strength as 1 cc. of the No. 1 solution.

The two solutions are placed in burettes of 50 cc. capacity, graduated in one-tenth cubic centimeter. In a laboratory where the solutions are to be used frequently, it is desirable to connect the burettes with bottles which contain a stock of each solution.

**Titration of the Gelatin.**—By means of a pipette measure out 10 cc. of the gelatin solution into each of four large test-tubes and label these 1, 2, 3, and 4.

To tube 1 add 2.5 cc. of the deci-normal solution.

To tube 2 add 2.8 cc. of the deci-normal solution.

To tube 3 add 3.0 of the deci-normal solution.

To tube 4 add 3.3 of the deci-normal solution.

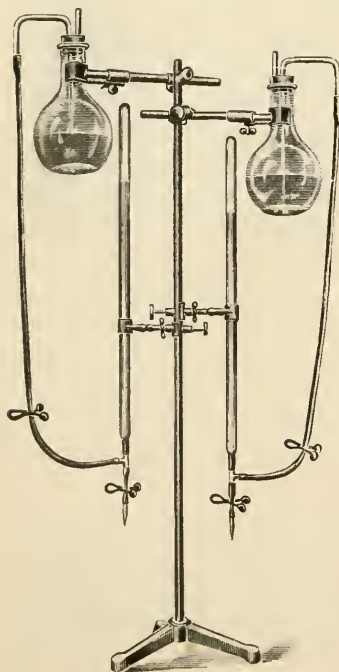


Fig. 2.

Heat each tube in the flame till the liquid boils, in order to expel the carbonic acid and to precipitate the albumen, phosphates, etc. Then drop into each tube a strip of blue and a strip of red litmus paper. These should be immersed in the liquid. Again, heat each tube to boiling and set aside for one minute. Then by means of a glass rod draw out the papers side by side onto the wall of each tube, and when cold compare the colors of the papers by holding the tubes before the window over a white surface. What was originally the blue paper, in tube No. 1, will probably show a red color. Therefore, 2.5 cc. of the deci-normal solution is not sufficient to neutralize the 10 cc. of gelatin. On the other hand, in tube four, both papers may be blue, indicating that 3.5 cc. is more than is necessary to neutralize. The neutral point, that is, where the red and blue papers retain their color side by side, lies therefore between the two extremes. Tube two may still show a slight acid reaction. Tube three may be neutral or nearly so. If the neutral point lies between 3.0 and 3.3 cc., it may be well to measure out fresh portions of 10 cc. of gelatin and to add 3.1 and 3.2 cc. of the alkali. The reaction should be tested as given. In this way it is possible to determine the neutral point to within one-tenth or two-tenths of a cubic centimeter, and this corresponds to a probable error of only one or two cubic centimeters of normal alkali per liter. Delicate litmus papers are of course desirable in this process and are better than a litmus solution.

The above experiment has shown that 10 cc. of gelatin requires, we will say, 3.0 cc. of deci-normal alkali for neutralization. In order to ascertain the amount of alkali necessary for the neutralization of all of the remaining gelatin, this must be measured in a cylindrical graduate. The amount left, we will say, corresponds to 950 cc. The amount of deci-normal alkali necessary to neutralize this quantity is ascertained from the following proportion:  $10 : 3 :: 950 : x$ , therefore  $x = 285$ .

That is to say, in order to neutralize the 950 cc. of gelatin, it would be necessary to add 285 cc. of the deci-normal alkali. Since this corresponds to 28.5 cc. of the normal alkali, the latter is added in preference to the large volume of the former, which would unnecessarily dilute the liquid.

Inasmuch as bacteria grow best on alkaline media, it is advisable to add an amount of alkali over and above that necessary to neutralize the medium. An excess of 10 cc. of normal alkali per liter imparts a desirable alkalinity. Hence to the 950 cc. of gelatin there will be added 28.5 cc. of normal alkali for neutralization, and 9.5 cc. for alkalinity, a total of 38 cc. of normal alkali.

After the addition of the necessary amount of alkali to the gelatin in the jar, the latter is then placed in the water-bath. The water is now raised to boiling and maintained at this point for three-quarters to one hour. The albuminous substances present in the meat extract now coagulate in flakes and clear up the liquid, so that on subsequent filtration it will be perfectly clear.

The liquid is then filtered through a plaited filter. This may be obtained ready made, and Schleicher & Schüll's No. 580 is particularly well adapted for filtering gelatin. It is advisable to pass some boiling water through the filter just before filtering the gelatin. If the first portion of the filtrate is cloudy it should be returned to the filter.

The filtrate should be: (1) perfectly clear; (2) slightly alkaline in reaction; (3) should not become cloudy or coagulate when boiled in a test-tube for one to two minutes; and (4) should solidify when cooled.

If the filtered gelatin answers the above requirements, it is ready to be "tubed," that is to say, filled into clean plugged tubes. The latter are frequently sterilized by heating in a dry-heat sterilizer for one hour at 150 degrees. This procedure, however, is not necessary, since sterilization of the cotton plug will be obtained by subsequent exposure to steam. This medium should be filled into the tubes by means of a small funnel, to a depth of one and a half inches. The utmost care should be taken to prevent touching the neck of the tube with the gelatin, since otherwise the cotton will adhere to the tube. The tubes of gelatin are then sterilized in steam for fifteen minutes on each of three consecutive days.

#### NUTRIENT BOUILLON.

A liter of meat extract is prepared according to the directions given above under gelatin. To this blood-red fluid ten grams of Witte's pepton and five grams of salt are added, and the mixture is warmed at 60 degrees till solution results. The liquid is then titrated according to the directions given. Portions of 10 cc. placed in each of four test-tubes receive 0.4, 0.6, 0.8, and 1.00 cc. of deci-normal alkali. After boiling the contents of each tube, red and blue litmus papers are immersed in the hot liquid. The neutral point is thus readily ascertained. It is usually reached by the addition of about 0.5 cc. of the alkali. The remaining liquid is measured, and the amount of normal alkali necessary to neutralize this entire quantity is then calculated. This calculated amount, plus 10 cc. of normal alkali per liter, is then added to the liquid.

The liquid is then placed in a flask, or in the enamelled jar, and immersed in a water-bath, which is then heated to boiling for about one-half hour. The liquid may be boiled directly over the flame, in which case, owing to the loss of water by evaporation, it is advisable to mark the level of the liquid at the beginning of the operation. A better procedure is to weigh the jar and contents before and after boiling, and for the number of grams lost in weight a corresponding number of cubic centimeters of distilled water are added.

The liquid is then filtered through a wet filter. Although the filtrate may be perfectly clear, it not infrequently happens that on subsequent sterilization a cloudiness or deposit forms. This can be avoided if the filtered liquid is concentrated by boiling to about one-half its original volume and then replacing the water lost by evaporation and filtering a second time. The clear bouillon is then filled into tubes and sterilized by steaming for fifteen minutes on each of three consecutive days.

#### NUTRIENT AGAR-AGAR.

A liter of bouillon is prepared as above, except that the second heating or concentration is omitted. The liquid is placed in a clean beaker or jar, and twenty grams of finely cut up agar are added. The jar and contents are weighed and placed over a flame and boiled. Agar dissolves better in clear bouillon than in a meat extract: moreover, the subsequent filtration is rendered easier because



of the presence of less foreign matter. The heat should be just sufficient to boil the liquid, otherwise it is liable to froth and run over. The liquid is boiled for one-half to three-quarters of an hour, or until the agar has completely dissolved. The jar and contents are now weighed, and distilled water is added to replace that lost by evaporation.

The jar is then placed in a water-bath which is kept at a temperature of 50 to 60 degrees C. for about two hours. The finely divided insoluble matter gathers into masses and settles to the bottom, leaving a clear, transparent liquid.

The filtration of a two per cent. agar through paper is usually a very slow, tedious, and unsatisfactory process. The author prefers filtration through absorbent cotton, inasmuch as this can be done rapidly and well. A liter of agar may be filtered in a few minutes, and frequently it is obtained perfectly clear without any deposit. At other times a very slight deposit may form in the tubes, which, however, interferes in no wise with the usefulness of the medium. The method employed is as follows:

The filtering arrangement is shown in Fig. 3.

The receiving flask ( $1\frac{1}{2}$  liter capacity) is connected with a Chapman aspirator. A large funnel provided with a rubber stopper is fitted to the flask. A Witte's perforated porcelain plate (10 cm. in diameter) is placed in the funnel and steadied in position by the glass rod which passes through the central opening. A disc of muslin having the same diameter as the plate (or a trifle less) is placed in the latter. Then a circle of absorbent cotton, about 11 cm. in diameter, is placed on top, and finally another layer of muslin. A second Witte's plate is now placed on top and remains there as a weight, only during the preliminary warming up of the funnel. About a half liter of boiling water is then poured into the funnel. The pump is set into action and the hot water rapidly passes through. It is returned to the funnel several times in succession, thus warming up the funnel, filter and flask. During the operation care should be taken to see that the cotton closes tightly over the edge of the porcelain plate and that no openings exist. While the pump is still active the upper plate is removed.

While the filter is still hot and the pump active, the well sedimented agar is slowly poured into the center of the filter. The liquid passes through as rapidly as it strikes the surface. The impurities are brought last upon the filter. If the filtrate is not perfectly clear, when examined in a test-tube, it should be passed through the same filter a second time. It is important that the agar be well sedimented previous to filtration. If this is not the case, the liquid will not pass through perfectly clear, and the filter, moreover, will tend to clog. If clogging occurs, the upper muslin layer may be carefully removed, in which case the filtration will recommence.

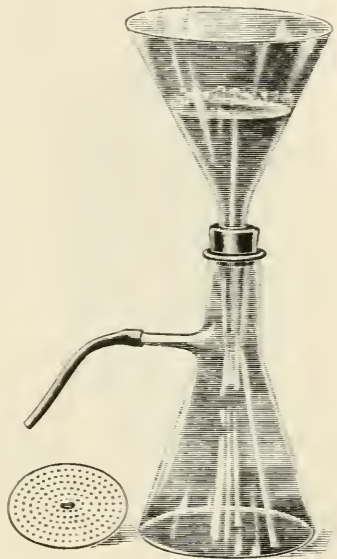


Fig. 3.



The filtered agar is then filled into tubes and sterilized in steam for thirty minutes on each three successive days.

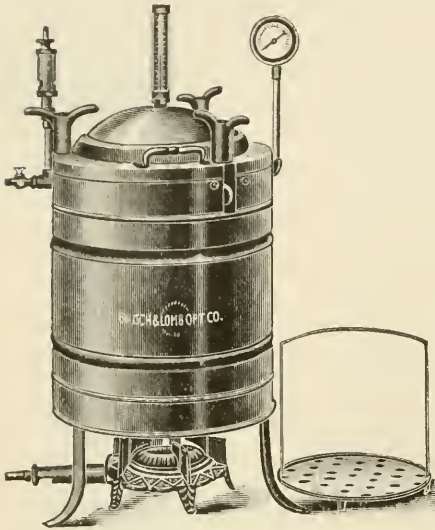


Fig. 4.

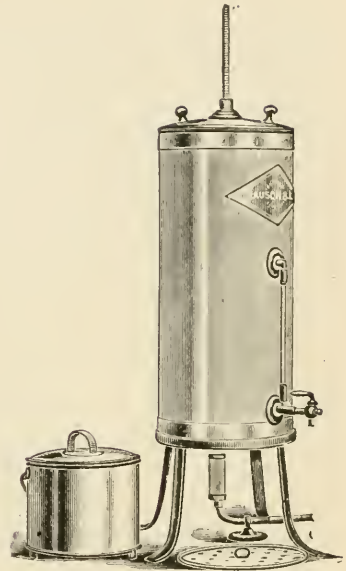


Fig. 5.

### STERILIZATION.

The tubed media are always sterilized in steam. If an autoclave (Fig. 4) is at hand, a single steaming at 115 degrees C. for fifteen minutes will be sufficient, otherwise it is necessary to resort to fractional sterilization, in which case the material is steamed on each of three consecutive days. This is done in order to allow the spores that may be present an opportunity to germinate. Various steam sterilizers are used for this purpose. The Koch sterilizer (Fig. 5) and that of Arnold (Fig. 6) are well known. In the author's laboratory, where there are seventy-five students at work at the same time, the use of these sterilizers has been found unsatisfactory. They have been replaced by small copper pails which are placed upon the water-bath.

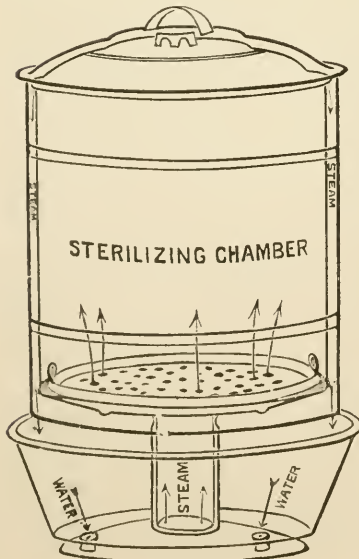


Fig. 6.

At a very small outlay, each student is provided with his own sterilizer. Consequently, no time is wasted in waiting either for a sterilizer or for the water to boil. For the details of construction, the reader is referred to this journal, vol. 1, page 33.

## NEWS AND NOTES.

CONCERNING FRESH WATER AQUARIA.—Mr. Wood's note, in the November number of the Journal, on the cultivation of algæ in aquaria, prompts the publication of a device we have used for several years past with very gratifying success and with a minimum of care. It is simply to cover the battery jars or other aquaria with loose covers; circular glass plates are excellent, which will exclude the dust, but permit free access of air. In such aquaria it very rarely happens that bacterial zoöglœa form on the surface, a trouble almost certain to occur if the aquaria are open.

By this simple means we have kept aquaria for as long as four years, without further care than to replace now and then the water lost by evaporation.

Among other algæ that thrive and fruit under this treatment are Chara, Nitella, some species of Spirogyra, Oedogonium, Coleochaeta, and Vacheria, though the last is rather fickle in its behavior.

Besides these, unicellular forms are always abundant, both plants and animals, and it is worthy of note that we have found amœba in great abundance in an aquarium that had been unchanged, save by the occasional addition of tap water, for nearly three years.

Before adopting this method with my aquaria, the collecting of fresh material for class work always consumed a good deal of time, as in open aquaria the plants would usually decay in a week or so; but since I learned the virtue of the covered aquarium, the keeping on hand of an abundance of fresh water algæ has been the lightest of tasks.

C. V. PIPER.

Agricultural College, Pullman, Wash.

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The University of Montana has been working under many difficulties since it started some four years ago. It was obliged to begin work in one of the public school buildings of the city of Missoula, and the work of the university has grown beyond the capacity of the building. However, two buildings are now about completed, and will be occupied before the first of March. For the work in Biology, tables have been planned for this particular work. They are twenty-eight inches high, twenty-eight inches wide, and four feet long. The tops are of oak, inch and a half thick, stained black and paraffined. On either side of the student, when he sits at the table, are two drawers and a space below for larger pieces of material. The tables thus will afford opportunity for work with two sets of students by working them at different days of the week, and give each student two drawers and one space below. The department of Biology has been organized but two years, and at first the class was small, consisting of but six. At the present time there is a very hopeful outlook for this line of work, and the department is as crowded with students as those that are older. In this short time some very good work has been done, and when the new quarters are occupied there will no doubt be much more interest than can be aroused in small rooms without opportunity for any but the more elementary lines of work.

M. J. E.

# Journal of Applied Microscopy.

L. B. ELLIOTT, EDITOR.

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\* \* \*

THIS being the first issue of the second year of the JOURNAL's existence, a considerable number of sample copies will be sent to persons who are not subscribers. If you, gentle reader, chance to be one of that number, accept this copy with our compliments, examine it thoroughly, and if it promises to be of interest to you, or helpful in your work, send in your subscription.

\* \* \*

MR. H. H. WAITE, of the Bacteriological department, University of Michigan, will take charge, beginning with the March number, of a department of Current Bacteriological Literature, to be conducted along the same lines as the departments now devoted to botany and animal biology.

\* \* \*

THE publication in each issue of the JOURNAL of "A Partial List of the Contributors" does not mean that these writers have engaged to supply all of the material to be published, but that they have signified their willingness to aid in maintaining a journal for applied microscopy, to make special efforts in order that it may be helpful to its readers. The usefulness of the JOURNAL depends largely upon bringing together in accessible form the results of the experience of a majority of the workers in every field in which the microscope is required. The time spent in putting on paper the results of investigation and experience is not only repaid in aiding many less favored in that particular line of work, but in adding to the permanent records of science, the accumulation of which will ultimate result in the simplification and perfection of microscopical technique. The JOURNAL is intended for all who use the microscope, and its development in any given direction is due largely to the demand for information on that particular subject. We depend largely upon the expressions of our readers to guide us, and criticisms and suggestions, as well as contributions, are welcomed and will receive careful attention.

## The Meeting of the Affiliated Scientific Societies.

The American Society of Naturalists, with its seven affiliated scientific societies, met in New York, at Columbia University, on December 28 and 29. The American Chemical Society met at New York on December 27 and 28, and the New York State Science Teachers' Association held its annual meeting at the Teachers' College, which is now under the management of Columbia University, December 29 and 30.

The meetings were attended by investigators and teachers from all parts of the country, and into the four days were crowded reports of investigations that the professors had carried on between working hours, experiments to demonstrate recent discoveries, and, among the teachers, discussions regarding the best way to develop more scientists.

In the intervals between meetings there were illustrated lectures, visits to the American Museum of Natural History, and to the New York Botanical Garden, receptions, and the annual dinner of the affiliated societies at the Hotel Savoy.

At a downtown meeting of the chemists, experiments were performed with liquid air, but as the gentleman declined to repeat the demonstrations at the university, many were unable to see them.

Among the interesting things on exhibition, was a collection of animal forms taken in Egypt during a search for material on which to work out the development of polypteris, and a live polypteris was also shown. It was announced that specimens from the Egyptian collection would be given, on application, to competent investigators for study.

The American women's table at the Zoölogical station of Naples was reported as being vacant. Applications for the table will be received by the secretary, Dr. Ida H. Hyde, No. 1 Berkely St., Cambridge, Mass.

Among so many meetings held at the same time, it was impossible to hear all the papers that one might wish to in the different sections.

Dr. H. S. Jennings reviewed his work on the "Laws of Chemotaxis in *Paramecium*." The question as to which activities of animals are purposeful, and which are merely mechanical responses to stimuli, has received considerable attention, and this study of the *paramecium* was made for the purpose of answering that question as far as possible, for a one-celled organism.

Different acids and alkalies were tried and the *paramecia* were found to be positively chemotactic toward weak solutions of acid, negatively chemotactic toward weak alkaline solutions, and negative to strong solutions of either. All the protective movements of *paramecium* depend upon negative chemotaxis. Positive chemotaxis is inactive and exists only as the absence of negative chemotaxis. A *paramecium* will swim in all directions until it comes in contact with a negative substance, which it will avoid, and when it has once entered a positive drop of fluid it becomes negatively chemotactic to its surroundings.

The negative reaction is always the same, the *paramecium*, by turning toward the aboral cilia, turns over and swims off, and these motions will be performed even if they result in carrying the organism into the negative fluid instead of out



of it. In that case the motion is merely repeated, and if immersed in the fluid the animal will continue to turn for ten minutes or more.

All negative stimuli produce the same reaction. No intelligence is shown.

Prof. Herrick added the observation that paramœcia raised in his laboratory near a radiator, for about one hundred generations, were positively thermotropic to a temperature of 70 degrees, 24 degrees being about the normal temperature.

Dr. S. Watase, in his paper on the "Characteristics of Mitosis and Amitosis," reviewed the literature of the subject up to the present date, and added the results of his own studies.

Mitosis may occur in many different ways. The spindle may originate inside of the nucleus and then migrate out, or it may originate outside of the nucleus. Great variation may be found in the same family. There may be division without a centrosome, or there may be a centrosome alone, or there may be neither centrosome nor spindle.

The characteristics of mitosis are neither the spindle nor the centrosome; these are only accessory, but the essential features consist in the special arrangement of chromatic substance in rods or chromosomes, and in increased nucleic acid. These changes always occur, while in amitosis the character of the chromatin is not changed at all and nucleic acid is not present.

He considers that the two types are distinct and independent, although they may exist side by side, as for example, in the fish egg, where the blastoderm may divide by mitosis while the periblast divides by amitosis. The karyokinetic cell no longer belongs to any tissue, as brain, muscle, etc., but is embryonic or protomorphic in character.

Amitosis is the homologue of fission and asexual reproduction, and in this the acquired characters would be retained by the new individual.

Mitosis is the homologue of sexual reproduction. Here acquired characters are eliminated and the new organism may take up any line of development.

A paper by H. W. Rand, on "Regeneration and Regulation in *Hydra viridis*," was read. Since the Hundred-headed *Hydra* of mythology, this animal has been renowned for its power of overcoming any inconvenience that might be caused by the loss of parts of its corporeal entity. From the experiments performed it was found that the upper half of the hydra was less active than the lower half in regeneration. Growth is apparently controlled by a law of symmetry, for the right number of tentacles is always regenerated whatever the mutilation may be.

The same thing was shown by Dr. A. E. Verrill in the starfishes. He had observed thousands of mutilated starfishes, and exhibited specimens showing the symmetrical regeneration of lost parts.

Dr. Verrill also showed specimens and drawings of the fauna of Bermuda, among which were actinians that place their ova in pockets on the outside of the body, where they develop. He called the attention of the society to the great desirability of establishing a Zoölogical station at Bermuda.

A paper by Prof. C. F. Hodge, on "Possible Amœboid Movements of the Dendritic Processes of Cortical Nerve Cells," was received with interest. Much attention has been paid to the condition of nerve cells in their relation to mental

and physical states, to know whether the chemical nature of the cells or the form of their processes changes during activity or rest.

The experiments were made to determine the condition of nerve cells in their sleeping and waking states. Puppies were used, and it was hoped that by cutting through the head quickly at one blow with a sharp, thin knife and allowing the parts to fall into the fixing fluid, the cells would be hardened before their processes could expand or contract in case any such change really did occur.

To determine the condition of nerve cells during rest, a puppy after sleeping all night was killed immediately upon waking, while for comparison its mate was left playing all day until thoroughly tired and then killed after sleeping a few moments.

The cells of the puppy that had slept all night showed the dendritic processes well expanded, while the cell processes of the puppy that had played all day were full of varicosities. This apparently contradicts the current theory that the cell processes are in contact during activity and that the contacts are broken during sleep, for the experiment shows that the varicose condition results from exhaustion of the cell, and that the dendritic processes become expanded during the resting condition.

Prof. Conklin discussed "Protoplasmic Movements as a Factor of Differentiation." He believes that the polarity of ova is not dependent upon gravity, but is due to flowing movements of the protoplasm and that the position of the centrosome and other organs of the cell is caused by these currents. He thinks it is insufficient to say that changes in position of different organs of the cell are brought about by chemotaxis, but these protoplasmic currents are the medium through which chemotaxis acts.

Prof. C. H. Eigenmann gave a paper entitled, "Further Observations on the Eyes of the Blind Fishes." He is working on the Amblyopsidæ, and told the society that during the summer he had crawled through nine caves and secured eight fishes of the type required. The forms in which the eye has not yet disappeared are more favorable for study. The fishes of this family are all negatively heliotropic, and the eye is used only for the purpose of avoiding the light. In those found outside of caves the retina is thin, while in inside forms it is thick, but in both cases the eye is degenerating.

The members of this family have a characteristic color pattern which extends in lines along the upper, middle, and lateral portions of the body and is related to the muscle bands. The pattern is always present, but with a varying degree of color, and the amount of color present is not affected by the light.

Mr. N. R. Harrington read a paper on the "Respiratory and Breathing Habits of Polypterus." He does not accept the view that the Dipnoi may be taken as ancestors of the air-breathing animals, because the special adaptations they present are not for breathing air suspended in water, but for enduring long periods of drouth by encasing themselves in mud.

Attention was called to "A Specific Case of the Elimination of the Unfit" by Dr. H. C. Bumpus.

The unfit in this instance was the ubiquitous English sparrow, which had been killed in large numbers during a severe ice storm. Measurements and weights of the sparrows were taken, and the comparison of the results showed

that there is a certain normal type for weight, length of wing, etc., that is best adapted to the preservation of the species, and that any variation from this type, either in increase or diminution of size or weight, lessens the sparrow's chances of living.

While these papers were being presented to different sections of the societies, the teachers across the street, at the Teachers' College, were earnestly considering what scientific facts should be taught, and how the subjects should be presented to students.

Although two years are spent on a single language—Latin—in the secondary schools, half a year is usually considered sufficient time in which to master a science, and in consequence the choice of subject matter that will give an adequate idea of the science becomes a difficult question.

In section A the question was raised as to whether biology should be taught as a recreation or as a science. Part of the educators were in favor of teaching biology from the standpoint of dissection and comparative anatomy, with bearings on the structural relations of different forms and upon the laws of life. Others preferred to awaken the interest of the student by the study of external forms and activities of animals, and to introduce little or no dissection.

Dr. Conklin advised all teachers to undertake some research and so keep in touch with the work of investigation. The progress of science has been due to the efforts of investigators, and the teacher's efficiency will be greater if he can take some part in this work.

Dr. C. B. Davenport of Harvard University presented a printed outline of the zoölogical requirements for entrance to the Lawrence Scientific School. The outline includes the study of twenty animal forms, eight of which belong to the Arthropoda. The outline is as follows:

- |                 |                         |
|-----------------|-------------------------|
| I. Grasshopper, | XI. Daphnia,            |
| II. Butterfly,  | XII. Slug,              |
| III. Beetle,    | XIII. Anodonta or Unio, |
| IV. Fly,        | XIV. Hydra,             |
| V. Starfish,    | XV. Paramecium,         |
| VI. Nereis,     | XVI. Frog's Egg,        |
| VII. Earthworm, | XVII. Smelt,            |
| VIII. Spider,   | XVIII. Newt,            |
| IX. Lithobius,  | XIX. Lizard,            |
| X. Crayfish,    | XX. Sparrow.            |

He considers that ten of these twenty exercises, thoroughly worked out, will be sufficient to occupy five periods a week for half a year, or three periods for one year.

He recommends that closely related types be studied in immediate succession, although, since living forms are required, this will depend upon the availability of material.

Dr. Davenport offers to give addresses of dealers to teachers who wish assistance in obtaining living material, or to answer questions concerning the work as laid out in the pamphlet.

Two of the exercises are here given :

## PARAMECIUM.

*Drawing.*—Whole animal, showing shape of body, cilia, vestibule, food globules, non-contracting vacuoles, contracting vacuoles, nucleus, trichocysts.  $\times$  about 200.

*Observations on the Living Animal.*—1. Is the protoplasm in the body of Paramecium quiet or moving? 2. Place Paramecium in a drop of gelatin solution (2.5 per cent. gelatin in water). Cover with a cover-glass, select a quiet individual, and observe how the carmine grains pass by it. Indicate by arrows placed outside the periphery of the drawing the direction of movement of the carmine. What do you infer concerning the movement of the cilia? Do the grains whirl as much about a moving individual as about a quiet one? Can you explain? Why are not all the Paramecia carried off by the current when the carmine is run under the cover-glass? 3. Place upon a glass slide a drop of water containing Paramecia. When they are uniformly distributed, put a few grains of common salt as the margin of the drop. After a few seconds observe the distribution of the organisms. How is the result to be interpreted? 4. Place upon the glass slide a drop of water containing Paramecia; alongside, but not in contact, place a drop of beef extract. Connect the two drops by a thread of water. After a few seconds observe the distribution of the organisms. How is the result to be interpreted?

*Topics for the Teacher.*—1. How the Infusoria get into the aquarium? 2. The habitat and food of Paramecium. 3. Allies of Paramecium; Stentor, Vorticella, Carchesium, Euglina, the Suctoria, Amœba, the Gregarinidæ. 4. Economic importance of Amœba and the Gregarinidæ. 5. Reproduction of Paramecium.

## BUTTERFLY.

*Drawings.*—1. Imago, dorsal view, wings expanded.  $\times$  1 or 2. 2. Imago, left side, wings closed. (The bodies in one and two are to be drawn parallel to each other.)  $\times$  1 or 2. 3. Imago, front of head,  $\times$  10. 4. Pupa, left side. 5. Full grown larva, dorsal view; full grown larva, left side.

*Questions on External Anatomy.*—1. How many segments behind the head in (a) the imago? (b) the larva? (c) the pupa? 2. What external organs of the imago can be identified in the pupa? 3. Which feet of the larva correspond to those of the imago?

*Observations on the Living Larva.*—Each student (or group of students) should be provided with a glass vessel covered with netting and containing food leaves, for keeping the larva during pupation. 1. How is locomotion effected? Illustrate by diagrams. 2. How does the larva feed? Observe and record the movements of the mouth parts and of the head during feeding. Draw the outline of a partly eaten leaf. 3. (This observation must extend through several days.) Make and record observations upon the act of pupation.

*Topics for the Teacher.*—1. The habits and food of butterflies. 2. The number of broods of butterflies during a single season, and seasonal dimorphism. 3. Protective resemblance and mimicry. 4. The larger divisions and commoner native forms of Lepidoptera. (Examples of Lepidoptera illustrating the common



native types should be shown and students encouraged to collect and classify them.) 5. The Hymenoptera; their structure, classification and habits.

## Papers Read at the Meeting of the Affiliated Scientific Societies, December, 1898.

### AMERICAN MORPHOLOGICAL SOCIETY.

W. PATTEN: Gaskell's Theory of the Origin of Vertebrates from Crustaceans.

BASHFORD DEAN: Notes on Myxinoid Development.

F. B. SUMNER: Notes on the Early Development of the Cat-fish.

J. REIGHARD: On the Development of the Adhesive Organ of *Amia*.

W. E. RITTER: On the Reproductive Habits and Development of the Californian Land Salamander, *Autodax* (presented by G. H. Parker).

C. H. MINOT: 1. Notes on Mammalian Embryology.

2. Professor O. van der Stricht's Researches on the Human Ovum, the Nervous System of *Amphioxus*, and the Development of *Thysanozoon* (demonstrations.)

S. P. GAGE: Notes on the Morphology of the Chick's Brain.

W. A. LOCY: Review of Recent Evidence on the Segmentation of the Primitive Vertebrate Brain.

C. J. HERRICK: Metameric Value of the Sensory Components of the Cranial Nerves.

W. A. LOCY: New Facts Regarding the Development of the Olfactory Nerve.

N. R. HARRINGTON and E. LEAMING: Action of Different Colors upon Protoplasmic Flow of *Amœba*.

C. H. EIGENMANN: Further Observations on the Eyes of the Blind Fishes.

U. DAHLGREN: The Breathing Valves of Teleost Fishes.

N. R. HARRINGTON: Respiratory and Breathing Habits of *Polypterus* (demonstrations).

G. H. PARKER and F. K. DAVIS: The Coronary Vessels in Fishes.

L. H. SNOWDEN: Notes on the Origin of the Blood Vessels and Heart in the Chick.

H. F. OSBORN: Vertebral Characters of the Herbivorous Dinosaurs, especially as shown in *Diplodocus*.

J. L. WORTMAN: The Evolution of the Cannon-bone in the Artiodactyla.

J. S. KINGSLEY and W. H. RUDDICK: The Ear Bones of the Mammalia.

C. O. WHITMAN: The Evolution of the Color-pattern of *Columba livia* from that preserved in *C. affinis* Blyth.

H. C. BUMPUS: A Specific Case of the Elimination of the Unfit.

A. E. VERRILL: The Marine Fauna of Bermuda with Reference to its Fitness for a Zoölogical Station (illustrated).

G. W. FIELD: The Anatomy of the Spermatozoön of Invertebrates (demonstrations).

J. H. MCGREGOR: The Middle-piece of the Urodele Spermatozoön.

H. E. CRAMPTON, JR.: The Origin of the Yolk in the Egg of *Molgula*.

E. G. CONKLIN: Protoplasmic Movement as a Factor of Differentiation.

S. WATASE: The Characteristics of Mitosis and Amitosis.

F. H. HERRICK: 1. A Case of Egg within Egg.

2. Abdominal Pregnancy with Histolysis of Fœtus.

W. R. COE: The Early Development of *Cerebratulus*.

C. B. WILSON: Regeneration and Fission in *Cerebratulus*.

H. S. PRATT: The Morphological and Physiological Significance of the Cuticula of Trematodes.

M. L. NICKERSON: Epidermal Glands in *Phascolosoma* (presented by T. H. Montgomery, Jr.).

A. G. MAYER: An Atlantic Palolo-worm.

W. S. NICKERSON: Notes on *Loxosoma Davenporti* (demonstrations).

H. S. JENNINGS: On the Laws of Chemotaxis in *Paramecium*.

C. B. DAVENPORT: Phototaxis of *Daphnia*.

- A. E. VERRILL: 1. Some Remarkable Variations and Monstrosities of our Common Starfish (demonstrations).  
 2. Several Curious Species of Actinians that Incubate or Carry their Eggs and Young attached to the body externally (illustrated).
- G. H. PARKER: Longitudinal Fission in Metridium.
- C. W. HARGITT: 1. Early Development of Pennaria Tiarella.  
 2. Grafting Experiments upon Hydromedusae.
- H. W. RAND: Regeneration and Regulation in *Hydra viridis* (presented by C. B. Davenport).
- W. M. WHEELER: Life History of *Dicyema*.
- F. H. HERRICK: *Hæmatococcus*.

## SOCIETY FOR PLANT MORPHOLOGY AND PHYSIOLOGY.

- DR. W. W. ROWLEE, Cornell University: The Morphological Characters upon which the Genus *Chamitea* is based.
- DR. J. W. HARSHBERGER, University of Pennsylvania: Some Peculiar Morphological Features of *Paulownia Imperialis*.
- DR. W. F. GANONG, Smith College: The Life-history of *Leuchtenbergia Principis* (abstract).
- PROFESSOR B. D. HALSTED, New Jersey Agricultural College: The Starch of Stomatic Guard-cells.
- MR. F. E. LLOYD, Teachers' College: Further Notes on the Embryology of the Rubiaceae.
- MR. CHARLES H. SHAW, University of Pennsylvania: The Inflorescences and Flowers of *Polygala Polygama*.
- MR. R. E. B. MCKENNEY, University of Pennsylvania: Observations on some Monocotyledonous Embryo-sacs.
- MR. R. E. B. MCKENNEY, University of Pennsylvania: The Structure and Relation of the Crystal Cells in Sensitive Plants.
- MISS EMELIA B. SMITH, University of Pennsylvania: The Structure and Parasitism of *Aphyllon Uniflorum*.
- DR. M. A. HOWE, Columbia University: On the Occurrence of Tubers in the Hepaticæ.
- DR. HENRY KRAEMER, Philadelphia College of Pharmacy: Morphology of the Genus *Viola*.
- DR. G. E. STONE, Massachusetts Agricultural College: Influence of Electricity upon Plants.
- DR. C. O. TOWNSEND, Maryland Experiment Station: Relations of Irritation to Growth.
- DR. ERWIN F. SMITH, Department of Agriculture: Sensitiveness of Certain Parasites to the Acid Juices of the Host Plants.
- DR. CARLETON C. CURTIS, Columbia University: Further Observations on the Relations of Turgor to Growth.
- DR. W. F. GANONG, Smith College: Some Appliances for the Elementary Study of Plant Physiology.
- PROFESSOR D. T. MACDOUGAL, University of Minnesota: Symbiosis and Saprophytism.
- PROFESSOR D. T. MACDOUGAL, University of Minnesota: Influence of Inversions of Temperature and Vertical Currents of Air upon the Distribution of Plants.
- DR. W. G. FARLOW (Presidential Address): Peculiarities of the Distribution of Marine Algae in North America.
- PROFESSOR H. F. OSBORN: Collections of Fossil Mammals and their Care.
- PROFESSOR G. F. ATKINSON, Cornell University: Notes on some Wood-destroying Fungi.
- PROFESSOR CONWAY McMILLAN, University of Minnesota: Some Notes on the Reproduction and Development of *Nereocystis*.
- DR. R. A. HARPER, University of Wisconsin: Preliminary Account of Sexual Reproduction in *Pyranema Confluens*.
- MR. T. A. WILLIAMS, Department of Agriculture: On the Morphology and Physiology of Certain Lichen Structures.
- DR. E. A. BURT, Middlebury College: The Formation and Structure of the Dissepiment in *Porothelium*.
- PROFESSOR D. P. PENHALLOW, McGill University: A Species of *Osmunda* from the Lower Cretaceous.
- DR. ERWIN F. SMITH, Department of Agriculture: Gelatin Culture Media.
- DR. CHARLES E. BESSEY, University of Nebraska: Notes on the Relative Infrequency of Fungi upon the Trans-Missouri Plains and the adjacent Foot-hills of the Rocky Mountain Region.

MESSRS. B. M. DUGGAR, Cornell University, and F. C. STEWART, New York Experiment Station: Different Types of Plant Diseases due to a Common Rhizoctonia.

MR. F. C. STEWART, New York Experiment Station: The Stem Rot Diseases of the Carnation.

#### AMERICAN PSYCHOLOGICAL ASSOCIATION.

MR. E. A. KIRKPATRICK: The Development of Voluntary Movement.

PROFESSOR E. B. DELABARRE: Report on the Effects of Cannabis Indica.

DR. DICKINSON S. MILLER: The Psychological Imagination.

PROFESSOR GEORGE T. LADD: Certain Hindrances to the Progress of Psychology in America.

MR. HENRY RUTGERS MARSHALL: Reason a Mode of Instinct.

PROFESSOR WESLEY MILLS: Nature of Animal Intelligence and how to Study it.

MISS MARY WHITON CALKINS: Psychological Classification.

PROFESSOR HUGO MUENSTERBERG (Address of the President): Psychology and History.

PROFESSORS JAMES, LADD, HIBBEN, MILLER, CALDWELL, and ARMSTRONG (discussion): The Relations of Will to Belief.

PROFESSOR J. MCKEEN CATTELL: Exhibition of Instruments for the Study of Movement and Fatigue.

PROFESSOR FREDERIC S. LEE: The Nature of Muscle Fatigue.

PROFESSOR HUGO MUENSTERBERG: The Physiological Basis of Mental Life.

PROFESSOR G. CARL HUBER: Observations on the Innervation of the Intracranial Vessels.

PROFESSOR G. T. W. PATRICK: Confusion of Tastes and Odors.

PROFESSOR C. F. HODGE (with MR. H. H. GODDARD): Possible Amœboid Movements of the Dendritic Processes of Cortical Nerve Cells.

DR. E. W. SCRIPTURE: Methods of Demonstrating the Physiology and Psychology of Color.

PROFESSOR G. W. FITZ: A New Chronoscope.

PROFESSOR OGDEN N. ROOD: On the Flicker Photometer.

MR. J. E. LOUGH: Changes in Rate of Breathing during Various Mental States.

DR. ROBERT MAC DOUGALL: Recent Investigations at the Harvard Laboratory.

DR. E. W. SCRIPTURE: Recent Investigations at the Yale Laboratory.

DR. JOHN P. HYLAN: Recent Investigations at the Illinois Laboratory.

DR. G. V. DEARBORN (Introduced by Professor Royce): Recognition under Objective Reversal.

DR. ARTHUR MAC DONALD: Further Measurements of Pain.

PROFESSOR W. A. HAMMOND: The Function of Will in Aristotle's Ethics.

PROFESSOR W. G. EVERETT: Ethical Scepticism.

PROFESSOR WILLIAM CALDWELL: Professor Baldwin's "Social and Ethical Interpretation."

PROFESSOR J. MARK BALDWIN: Genetic Determination of the Self.

DR. G. TOSTI (Introduced by Professor Baldwin): Art in the Light of Modern Psychology.

PROFESSOR CHARLES H. JUDD: A Study of Geometrical Illusions.

MISS MARGARET WASHBURN: Subjective Colors and the After Image.

MRS. CHRISTINE LADD FRANKLIN: Three New Cases of Total Color Blindness.

PROFESSOR GEORGE T. LADD: A Color Phenomenon.

E. M. B.

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NOTE—The statement in the December Journal that the division of nerve cells had been observed, was due to a typographical error. *No* should be read in place of *the* in line 39, page 219. An error in printing also occurs in the next paragraph. It should read, "attention is called to the importance of the question whether a genetic connection may be established between this so-called centrosome, and that which exists during cell division." In the title read "*two* Polychæte Annelids" instead of "*the* Polychæte Annelids."

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Wager, H.** The Nucleus of the Yeast Plant. This paper is introduced by a review of the literature of the subject from the time of Nägeli up to the present. The writer recommends as a fixing agent a saturated solution of corrosive sublimate, which should be allowed to act for at least twelve hours. Gram's solution of iodine was also of immense value. It should act for twenty-four hours. The material is then washed successively in water, 30 per cent. alcohol, 70 per cent. alcohol and methyl alcohol. A few drops of the alcohol containing the material is placed on a cover, and, when nearly dry, a drop of water is added. After the yeast cells settle, the water is drained off and the cells are allowed to dry up completely. The cover or slide with its layer of cells is then placed in water for a few seconds, after which it is ready for staining. The combination of fuchsin and methyl-green, or fuchsin and methylene blue, proved more effective than the carbol-fuchsin recommended by Janssens and Leblanc. The stained cells were mounted whole in glycerine or balsam, but material was also imbedded in paraffine for microtome sections.

A few of the conclusions are as follows: All yeast cells contain a nuclear apparatus. In the earlier stages of fermentation this consists of a nucleolus, in close contact with a vacuole which contains a chromatin network resembling that of the nuclei of the higher plants. In later stages of fermentation this vacuole may disappear, its place being taken by a granular network. The nucleolus is present in all cells. In the process of budding, the nuclear apparatus does not show any well defined stages in karyokinesis, and the division should probably be regarded as direct. The nucleolus divides either in the neck joining the bud to the parent cell or, more rarely, in the parent cell itself. One of the products of division passes into the bud. Several of the figures in the plates bear at least a superficial resemblance to the dividing nuclei of some of the lowest animals.

C. J. C.

**Debski, B.** Weitere Beobachtungen an *Chara fragilis*. Jahrb. f. wiss. Bot. 32: 636-670. pl. 11 and 12, 1898.

Debski has shown, in a previous paper, that in *Chara* there is no reduction in the number of chromosomes in any of the mitoses leading to formation of the spermatozoid. In the present article he finds that no reduction takes place in the development of the egg-cell. After the breaking down of the nuclear membrane, but before the formation of the spindle, protoplasmic radiations appear around the nuclear cavity. He believes that the spindle is at first multipolar. The cell plate arises from thickenings of the connecting fibers. In the nuclei of nearly all mature cells there occur in the nucleoli and nuclear network changes which often lead to fragmentation. Such cells are no longer capable of division.



The membrane of the Characeae usually shows no cellulose reaction with chlor-iodide of zinc or iodine and sulphuric acid.

Some of the figures appear stiff and diagrammatic, but on the whole they are easily understood.

C. J. C.

**Stevens, F. L.** The effects of aqueous solutions upon the germination of fungus spores. Bot. Gaz. 26: 377-406, 1898.

This article tends in a direction which, if pursued, will put the whole matter of fungicides upon a firmer basis.

Exact determinations were made for a number of acids and salts of the concentration needed, to prevent germination in a number of species of fungi. All solutions used were based on the normal strength, i. e., one gram molecule per liter of water; and cognizance was taken of the more recent theories regarding the ionization of the molecule. In general this theory received corroborative evidence from this work; for example, all copper salts were equally poisonous *per molecule*. Potassium cyanide was very slightly poisonous to the spores, notwithstanding its great poisonous action upon animals.

Since at the extreme dilutions used, ionization was complete, the effects and the ions themselves may be deduced. Thus, mercury, copper, hydrogen, and hydroxid ions are among the most poisonous, mercury leading all these in its poisonous activities. The ions of chlorin, iodine, sodium, potassium, etc., are non-toxic.

From the evidence deduced, the Bordeaux mixture contains much more copper than is needed. Spores of different species and different spores of a given species do not present the same resistance. *Uromyces*, for example, is much more resistant than *Macrosporium*.

In general, the substances showed an apparent agreement between the toxic power and the atomic weight.

OTIS W. CALDWELL.

**Lawson, A. A.** Some observations on the development of the karyokinetic spindle in the pollen-mother-cells of *Cobea scandens*. Proc. Cal. Acad. Sci. III. I: 169-184. pl. 33-36, 1898.

It was the purpose of this work to investigate the earliest stages in the development of the kinoplasmic fibers of the multipolar spindle.

Flemming's mixture, chromic-osmic-acetic strong solution was used for fixing, and the safranin-gentian violet-orange combination was used for staining. Other fixing agents and stains were used, but did not give as good results. Bergamot oil was used to precede the infiltration with paraffin.

The cytoplasm of the resting pollen-mother-cell presents a clear, uniform appearance, but as division approaches, a zone of granular substance accumulates about the nucleus. This zone is so constant that the writer proposes to designate it by a new name, perikaryoplasm. The perikaryoplasm was observed in living cells, and so could not be regarded as an artefact. When the nuclear wall breaks down, the perikaryoplasm and the linins of the nucleus form a network of kinoplasmic fibers which grow out into several projections. These projections become the cones of the multipolar spindle. Spindle fibers are formed by the elongation of the meshes of the network. The cones elongate, become sharp pointed, and then fuse in two groups to form the bipolar spindle. The mature spindle is characterized by the great length and crossing of the mantle

fibers. Spindle formation in the second division of the pollen-mother-cell is the same as in the first. No centrosomes were observed. The writer accepts the conclusion of the Bonn school, that centrosomes take no part in the formation of the spindle, but does not suggest any explanation for the transformation of the multipolar spindle into the bipolar.

C. J. C.

**Ikeno, S.** Untersuchungen über der Geschlechtsorgane und den Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb.f.wiss. Bot. **32**: 557-602, pl. 8-10 and 2 autotypes, 1898.

This important paper has been long promised, and the preliminary notes have been so often quoted that it does not seem new to connect Ikeno's name

with *Cycas*. The various stages in the development of endosperm and the formation of archegonia correspond closely with the conditions already described in other Gymnosperms. The development of the egg cell was traced in detail. The material for its increase in volume is a half-liquid protein stuff furnished by the nuclei of the wall cells and also by the nucleus of the egg itself. During the ripening of the egg, a ventral canal cell is cut off. The karyokinesis concerned in the cutting off of this cell appears to be of the heterotypic type.

The pollen grain consists of two small cells, which he calls prothallial cells, and one large embryonal cell. The latter is the cell which forms the pollen tube. The inner prothallial cell divides to form a stalk and generative cell, while the prothallial cell is resorbed. It would seem to the reviewer that it would be more appropriate to call the embryonal cell a "tube cell" and to restrict the term, prothallial cell, to the single cell which is resorbed. Two centrosomes appear in the body cell, and when this divides, each of the resulting spermatids receives a single centrosome. These centrosomes, which reach a diameter of  $15\mu$ , behave very much like those of *Gingko*, becoming drawn out into a band which generally makes four spiral turns and gives rise to the cilia.

Shortly before fertilization, the egg cell shows a crater-like receptive spot at its apex. After entering the egg, the spermatozoid nucleus slips out from its cytoplasm, which then disorganizes within the egg cell. The sperm nucleus unites with the egg nucleus at the receptive spot, and the fusion nucleus then presses deeper into the egg, *where it becomes dissolved*. It will be recognized that this is a peculiar and hitherto unknown type of fertilization, and one which needs abundant evidence to insure its acceptance.

The formation of the embryo corresponds closely with that already described for *Gingko*.

C. J. C.

**Jeffrey, E. C.** The Gametophyte of *Botrychium Virginianum*. Trans. Canadian Institute. **5**: 265-294. pl. 1-4, 1896-7.

These investigations are by far the most important of recent contributions to the morphology of the Ophioglossaceæ.

Hitherto the gametophyte of *Botrychium* was little known. All attempts at culture from the spores had failed to obtain more than two or three cells, and our knowledge was based on descriptions of a very few chance specimens. Mr. Jeffrey was so fortunate as to find over six hundred specimens at one time, and has since collected more. He finds that the prothallia are tuberous in form, destitute of chlorophyll, and entirely subterranean. The lower half of the gametophyte body, excepting two or three layers of cells, is infested with an intracel-

lular, endophytic fungus nearly allied to *Pythium*. The sexual organs are borne upon a median ridge on the upper surface of the prothallium. The antheridia resemble those of Eusporangiate Pteridophytes in general, while the archegonia show an approach to those of the Leptosporangiate Filicineæ. Though the young embryo passes through an octant stage, its primary organs, on account of their late appearance, can not be assigned to definite quadrants of the segmented oospore. The gametophyte does not end its existence with the production of a sporophyte, but persists for a long time, even for several years. The only gap in the life history which now remains to be filled is that between the three-celled, chlorophyll-bearing prothallia, obtained by Prof. Campbell, and the mature, saprophytic form now so accurately described and figured by Prof. Jeffrey.

Chicago.

W. R. SMITH.

**Ellis, W. G. P.** A method of obtaining material for illustrating smut in Barley. *Ann. Bot.* **12**: 566-567, 1898.

A supply of smutted barley may be obtained by sowing soaked, skinned barley that has been plentifully covered

by *Ustilago* spores. Stages in development of spores may be easily traced in such material. Freehand sections of ears about three-eighths of an inch long give a fair view of the mycelium and spore clusters. If more accurate study is desired, they may be removed and fixed in Flemming's or von Rath's solution. If smutted ears be removed and kept floating on water, the spores continue to develop and very often germinate.

It will pay teachers to remember this, for old and dry material is always more or less unsatisfactory to work with.

C. J. C.

**Errara, L.** Structure of the yeast cell. *Ann. Bot.* **12**: 567-568, 1898.

The principal conclusions are as follows: "(1) A relatively large nuclear

body exists in each adult cell. (2) Young cells contain no such body; a little later the old nuclear body divides, and one of its two daughters wanders through the narrow connecting channel into the young cell. (3) After division is complete, the two cells are still kept together by a mucilaginous, neck-shaped pedicel, which may or may not persist, thus explaining the occurrence of cell chains or isolated cells in different races of yeast."

C. J. C.

#### RECENT LITERATURE.

**Abel, R.** Ueber einfache Hilfsmittel zur Ausführung bacteriologischer Untersuchungen in der ärztlichen Praxis. 32 pp. Würzburg (A. Stuber), 1898.

**Bowhill, T.** Manual of bacteriological technique and special bacteriology. 296 pp. 100 orig. illus. London (Olliver & B.), 1898.

**Goebel, K.** Organographie der Pflanzen, insbesondere der Archegoniaten und Samenpflanzen. Zweiter theil: Specielle Organographie. I. Heft: Bryophyten. 8vo. pp. 283-385. figs. 131-359. Jena: Gustav Fischer, 1898. M. 3.80.

**Hartog, M.** Alternation of Generations. *Ann. Bot.* **12**: 593-594, 1898.

**Klebs, Geo.** Alternation of generations in the Thalophytes. *Ann. Bot.* **12**: 570-583, 1898.

**Lang, W. H.** Alternation of generations in the Archegoniata. *Ann. Bot.* **12**: 583-592, 1898.

**Lind, K.** Ueber Eindringen von Pilzen in Kalkgesteine und Knochen. *Jahrb. f. wiss. Bot.* **32**: 603-634, 1898.

**Oltmanns, Fr.** Zur Entwicklungsgeschichte der Florideen. *Bot. Zeit.* **56**: 99-140, 4 pl., 1898.

**Pearson, H. H. W.** Anatomy of the seedling of *Bowenia spectabilis*. *Ann. Bot.* **12**: 475-490. pl. 27 and 28, 1898.

**Wisselingh, C. van.** Ueber den Nucleolus von *Spirogyra*. Ein Beitrag zur Kenntniss der Karyokinese. *Bot. Zeit.* **56**: 195-226. pl. 10. 1898.

## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
 Agnes M. Claypole, Sage College,  
 Ithaca, N. Y.

## CURRENT LITERATURE.

**Leidig, F.** Vascular Epithelium. Arch. fr. Mikr. Anat. 52: 1898, pp. 152-155. Capillaries have been recently shown by Maurer to exist in the buccal epithelium of amphibia. Leidig noticed such in his work, "Die Zelle und Gewebe" (1885). He now calls attention to the existence of vascular epithelium in the earthworm and leach, and quotes the observations of various investigators on the subject. The superficial emergence of blood vessels is important in reference to the respiratory functions of the animals. A. M. C.

**Gardner, M.** Histogenesis of Elastic Tissue. Le Physiologiste Russe, 1898, pp. 3-14. From studies in the amniotic membranes of various mammals, the author reaches the following conclusions: 1. The elastic substance forms centrally in the protoplasm of cells, in granules.

2. The granules fuse and form delicate filaments, and this fusion may occur in the limits of one, all, or in several anastomosed cells. The nucleus plays no part, so far as known, in the formation or fusion of granules; this is probably a cytoplasmic process.

3. Delicate elastic filaments of adjacent cells fuse and form larger filaments, these form similar filaments, and together they form a larger fiber.

4. No active intrusion of elastic filaments into extra protoplasmic substance is observable. The formative cells determine the final reticular or fibrous structure. Nothing is said of the fate of the nucleus of the cell-forming fibers. A. M. C.

**Brachet, D. A.** Researches on the Development of the Heart and Great Vessels and the Blood in Urodel Amphibians (Triton alpestris). Archiv d'Anatomie Microscopique, 2, Fascicule, 2, 1898, pp. 251-304.

The results of the investigations are stated in the following summary:

1. The elements at whose expense the first rough outline of the heart and great vessels of the embryo are made are not migratory cells, which become masked at the point of the differentiation of the organ.

2. The heart and primary vessels have in Triton an origin clearly and exclusively hypoblastic.

3. The development is absolutely independent of that of the red blood cells as at one time they are hollow and empty; only secondarily, after they become connected with the blood islands, do they become filled with blood.

4. The blood has its origin at the cost of the yolk hypoblast, in a well defined region that is called the medio-ventral blood islet and its two branches of bifurcation. In the Triton this islet is the only spot of formation of the blood cells.

The following facts are shown by the author to be of great significance: Goette twenty-five years ago maintained that it was possible to establish a comparison between the vascular yolk structures of amphibians and of those vertebrates



having eggs with large quantities of yolk, birds for example. This comparison is perfectly justifiable. In amphibians the blood islet, with that portion of hypoblastic yolk which it occupies, is comparable to the area vasculosa of vertebrates with meroblastic eggs.

The analogies between the two are numerous. In all vertebrates in which the vascular area exists, it is the center of the first formation of blood. In the amphibian, the blood islet, occupying the individual surface of the hypoblastic yolk, is not only the first but the exclusive place of blood formation.

Another comparison, yet more interesting, is that in the Amphibia, as in the vertebrates having the area vascula, the vascular system at large and the blood in the blood islet form independently of the heart and chief embryonic vessels and at first have no connection with the former structures. Secondly, this connection is established.

Yet another point is that the midcentral blood islet and the part of the vitellin veins forming from it appears to be of value as an extra embryonic vascular system comparable with the vascular yolk apparatus of reptiles and birds. It is comparatively easy to explain the primitive independence of the vascular area and the body vessels of the embryo in vertebrates having the embryo lying spread out over a very abundant nutritive yolk, but in the amphibians, where the quantity of nutritive yolk is not only less in amount, but also distributed in all cells forming the embryonic arc, this arrangement is less comprehensible.

Many other points of great interest and great value are brought out by the author. Questions now open in embryology about this most difficult and vexed question are discussed, and the paper is one of great value to those interested in this subject.

A. M. C.

**Nemec, Dr. B.** Centrosome and Nucleus. The author compares the cell divisions in which the centrosome functions with those in which it seems to be absent, as in higher plants. No doubt exists as to the essential resemblance, and the author shows that in a cell without a centrosome the nucleus serves the same purpose in the prophase and the anaphase. The centrosome is really, in such cells, homodynamous with the nucleus before and after nuclear division.

A. M. C.

**Arnold, Prof. J.** The Structure of Cytoplasm. Prof. Arnold examined many kinds of cells in reference to the structure of their substance. In many cases, as in leucocytes and bone marrow, the plasmasomes are present and are bound by thread-like or rod-like processes into systems, sometimes reticular, sometimes spongy. The plasmasomes enclose granules (somatia) variously disposed, and a hyaline substance (paraplasm) in the interstices of the system. A great variety is possible in the structure of these systems with these materials.

In nervous tissue Arnold describes a general occurrence of rows of granules. In ganglion cells at least two different substances forming systems of granules are set apart from the hyaline interstitial substance. It may be that the one system composed of neurosomes has to do with conduction, while the other composed of plasmasomes has to do with nutrition.

A. M. C.

# Journal of Applied Microscopy.

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## General Methods in Botanical Microtechnique.

### II.

#### 7.—CUTTING SECTIONS.

The sections must be cut on a microtome. Cut one of the objects with a suitable amount of paraffin out of the cake by means of a sharp scalpel, taking care that the edges of the block will be parallel with the general contour of the object. Trim the block down to a rectangular shape and fasten it to a block of wood, or a special holder which goes with some microtomes. Before attempting to fasten the block to the holder, have the top of this covered with a cushion of paraffin. The paraffin block must be fastened firmly, and the edges especially sealed with a hot needle so that there will be no danger of having it come off. After having cooled off the block in cold water and trimmed the sides to be parallel, fasten it into the clamp of the microtome and adjust the knife and clamp so that the knife will strike the paraffin block perfectly parallel. The ribbon of sections should be straight and not coiled. If the ribbon coils, no good mounts can be made even if everything else has been satisfactory so far. The desirable thickness of the sections depends somewhat on the nature of the material and the object to be attained. As a general rule most sections may be cut ten microns ( $\mu$ ) thick. The section knife or razor must be sharp and clean, with no trace of the smallest notches, at least in that part with which the cutting is done. It is well to examine the edge of the knife under the low power of the microscope to see that it is in good condition. After the ribbon has been cut care should be taken to have all the pieces arranged in a continuous series, from left to right, on a clean sheet of paper. The sections may be covered with a wide bell jar. If the sections do not hold together well while cutting, the paraffin may be too cold or there may be other defects. These should be discovered and removed before proceeding further. Ribbons should be cut yards in length, without a single break, when serial sections are cut.

#### 8.—MOUNTING.

1. Take a clean slide and put a small drop of albumen fixative on it. Spread it out over the surface with the finger into a very thin, even layer, being careful that no part of the finger touches the slide before being covered with a layer of the albumen. The layer must be quite thin so that you can just leave a

noticeable impression of your finger on it. Too much albumen will ruin the preparation. The albumen fixative is made as follows:

1. 25 cc. of the white of a fresh hen's egg.
2. 25 cc. of glycerin.
3. 0.5 gram sodium salicylate.

Shake well and filter. This will keep well for at least six months.

2. Now lay the slide down on the table and put a few drops of distilled water on it, on top of the albumen film. Care must be taken here that the water will not flow over the edge of the slide.

3. Cut the ribbon into suitable lengths, according to the size of the square or oblong cover-glass, discarding the ends of the ribbon which do not contain sections. With a scalpel lay the pieces of ribbon on the water in the center of the slide in such a manner that one may begin at the upper left-hand corner and follow the sections in lines, as one reads the words on this page. Allowance must always be made for a certain amount of stretching of the ribbons when they are heated, as they are always more or less ruffled. Never press the sections down with the finger or by any other means, else the fine structure will be broken and distorted.

4. Warm the slide gently by putting it on the paraffin oven until the heat has straightened out the sections on the water, but do not let the sections get so hot as to melt the paraffin. The slides may now be placed on wooden blocks, which may be kept constantly on top of the oven for this purpose. It is best to let them remain for about twelve hours, when the water will all be evaporated and the sections firmly dried to the slide. Four, eight, or more slides can be carried through at one time just as well as a single one.

#### 9.—STAINING.

The sections are now ready for the staining. One must have the following Stender dishes:

1. Filled with turpentine.
2. Filled with xylol.
3. Filled with absolute alcohol.
4. Filled with 95 per cent. alcohol.
5. Filled with 85 per cent. alcohol.
6. Filled with 70 per cent. alcohol.
7. Filled with 50 per cent. alcohol.
8. Filled with 25 per cent. alcohol.
9. Filled with distilled water.

The various stains used may also be kept in Stender dishes if no special staining dishes are at hand. The following stains are recommended for general purposes:

1. *Anilin safranin*, alcoholic (50 per cent.) solution, made by combining equal parts of anilin water and a saturated alcoholic (95 per cent.) solution of safranin. The anilin water is prepared by shaking up anilin oil in distilled water. About 3.5 per cent. of anilin oil will be taken up by the water.

2. *Gentian violet*, a 2 per cent. aqueous solution.

3. *Iron alum*, a 2 per cent. aqueous solution of ammonio-sulphate of iron.

4. *Haematoxylin*, a 0.5 per cent. solution obtained by dissolving in hot water.

The remaining Stender dishes will therefore be as follows :

10. Filled with anilin safranin.

11. Filled with gentian violet.

12. Filled with iron alum.

13. Filled with haematoxylin.

#### FIRST STAIN—ANILIN SAFRANIN.

1. Melt the paraffin around the sections of two slides by heating them to 52° C. in the paraffin oven.

2. Wash off the paraffin by putting the two slides back to back into the Stender dish with the turpentine.

3. Transfer to Stender dish of xylol.

4. Next put them in succession into the dishes with absolute alcohol, 95 per cent., 85 per cent., 70 per cent., and 50 per cent. Let them remain in each one about ten seconds, more or less. Do not leave the dishes uncovered longer than necessary.

5. Transfer the slides from the 50 per cent. alcohol to the anilin safranin dish, and let them stain from two to four hours.

NOTE.—In taking sections from xylol to any stain, always pass down the grades of alcohol until the sections are in the same grade as the stain, and then transfer to the stain.

6. When the sections are stained wash them successively in the 50 per cent. alcohol, 70 per cent., 85 per cent., 95 per cent., and absolute alcohol. Judgment must be used as to how fast the transfer is to be made from one grade of alcohol to the other. They must generally be taken quite rapidly, as the alcohol will take out such stains as safranin.

7. Clear the sections by transferring them to the xylol. The sections must be thoroughly cleared. Leave them in xylol until they look transparent.

8. Take one slide out of the xylol at a time; wipe off the xylol with a clean rag, wiping quite close to the sections, but do not touch the sections.

9. Put a drop or so of Canada balsam (dissolved in xylol) on the sections at one side.

10. Put on a clean cover-glass in the following manner: holding the cover-glass with the edges between the thumb and forefinger, bring it down slowly and obliquely upon the drop so that one edge of it is first wetted by the balsam; and supporting the opposite edge with a needle, let the cover gradually settle down and spread out the balsam. There should be no air bubbles and just enough balsam to come to the edge of the cover-glass. Care must be taken to not let the sections become dry at any stage of the foregoing process. The slides may now be laid aside into a convenient place to dry. They may be studied immediately if handled with care for a few weeks until the balsam has thoroughly hardened around the cover-glass. If any balsam should get on the hands or instruments, it can easily be removed with a little xylol.



## SECOND STAIN—ANILIN SAFRANIN, GENTIAN VIOLET.

This makes a good double stain for many purposes. Stain first in the anilin safranin from two to four hours; then wash in 25 per cent. alcohol; next in water; and then stain from one to four minutes in the gentian violet. After washing in water, pass through the grades of alcohol, clear in xylol or clove oil, and mount in balsam.

## THIRD STAIN—HEIDENHAIN'S IRON-ALUM-HÆMATOXYLIN STAIN.

Run the slides down to water, and from this transfer to the iron-alum. Keep the sections in this from two to four hours, and after washing well in tap water, stain for twelve hours (or over night) in the hæmatoxylin. After this wash the slides again in water and wipe them clean, and as close to the sections as is safe. The sections are now black and must be cleared. To do this they are placed again in the iron-alum, which gradually takes out the excess of stain. They must be closely watched and examined from time to time under the low power of the microscope. When of a light greyish-blue color they are washed again very thoroughly in tap water so that all iron salt is removed, and are then carried through the grades of alcohol, cleared in xylol, and mounted in balsam.

## FOURTH STAIN—ANILIN SAFRANIN, IRON-ALUM-HÆMATOXYLIN.

After one has become accustomed to use the foregoing combinations successfully, the following is well worth trying. Stain first in anilin safranin or in anilin safranin and gentian violet, as described above; wash in water; and then stain in the iron-alum-hæmatoxylin according to the directions given, just as though the sections had not been stained at all. After staining, removing excess of stain, and washing in tap water, pass through the grades of alcohol, clear in xylol, and mount in balsam. This is one of the clearest stains I know of.

There are many other excellent combinations and stains, all of which should be learned gradually and employed. The person who uses but one method of killing and staining without trying others and comparing with them, may get certain results, but such results are always to be taken with some reserve. The stains mentioned above, it is believed, will give quite uniform and satisfactory results for general purposes, and they may be taken as the starting point for acquiring a technique which is both elaborate and extensive.

JOHN H. SCHAFFNER.

Ohio State University.

## Carbolic Acid as a Clearing Agent.

This is particularly useful in mounting marine diatoms *in situ* on seaweeds. Algæ covered with the frustules of *Rhabdonema arcuatum* and similar diatoms may often be found tossed up by the storms on the British coasts. To remove air from the interior of the frustules, drying and subsequent soaking in benzine have been recommended, but are only partially successful, many of the diatoms remaining filled with air. Soaking in water and passing successively through alcohol and oil of cloves will of course remove all air, but will often bleach the algæ on

which the diatoms grow, notably should these be *green* algæ. By the use of carbolic acid, into which the specimens are transferred directly from the water, this bleaching effect is largely obviated and better preparations obtained. As the object is, however, to show the attached diatoms, any contraction or slight injury to the color of the host, arising from the action of the carbolic, is of slight importance, and is, as a matter of fact, not sufficient to prevent a very pretty slide from being obtained.

Of course this method does not show the endochrome, but the markings on the frustule are distinctly seen, as well as its mode of attachment to the alga. I have mounted a quantity of material of *Rhabdonema arcuatum* by this method.

G. H. BRYAN.

University College of North Wales, Bangor, Great Britain.

## An Interesting Abnormality.

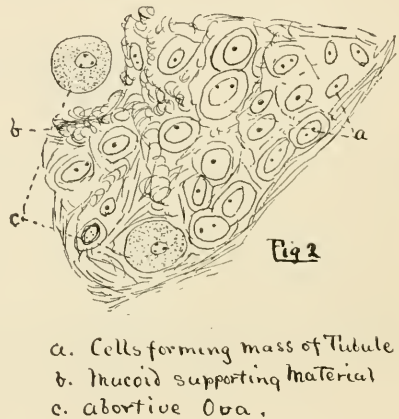
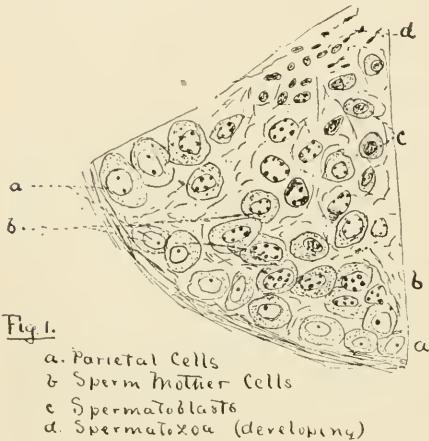
I wish to give a brief account of a peculiar abnormality which has recently come under my notice, hoping that some readers of this magazine may have found a similar condition or perhaps will do so in the future. In a large adult cat the left testis was retained in the abdomen, the right being perfectly normal. The retained gland was striking for its relatively small size and its peculiar position and relations. At first glance it seemed that this organ must be an ovary; its position, vascular supply, and size all tended to produce this impression, suggesting of course that the animal was hermaphrodite. Closer examination showed the structures to resemble those of the testis more nearly, but a microscopic examination was necessary to determine the point fully. Owing to the high abdominal position of the testis (it was just a little caudad of the kidney) the characteristic loop of the vas deferens over the ureter was lacking, although both efferent ducts opened as usual into the urethra. Along the length of the left duct, as far as the bladder, there was a broad peritoneal fold, attaching it to the abdominal wall and entirely preventing any slipping of the organ to an external or even lower abdominal position. In any case there was no fold of skin corresponding to the scrotal sac to receive it. These facts sustain the opinion that the abnormal position was an entirely constant one in the animal, and not one either accidentally or temporarily acquired.

On examining embryo kittens to find if possible at what stage the arrest in descent had occurred, it was found that the position corresponded closely to that found in an embryo of nine and one-half centimeters long, with the hair just beginning to develop. In these animals the descent of the testes into the scrotal sac takes place only a few days before birth, but they lie low in the abdomen some time before. So that the high abdominal position represented a somewhat early stage.

The two testes were removed, embedded in collodion and sectioned, and on examination the following facts were shown: the right testis was normal, spermatozoa were developing in the tubules, and all stages of the process were to be seen (Fig. 1). The outer layer of parietal cells (*a*) showed little chromatin in the nuclei, the cytoplasm was finely granular; the next layer of sperm mother

cells (*b*) had nuclei loaded with chromatic material, many of them showing karyokinetic figures of active division. On the inner surface of the tube were spermatoblasts and spermatozoa in all stages of development.

In the retained organ a widely different condition was evident; the tubules were smaller, not more than one-half to two-thirds the size of those in the right testis; the cells showed no gradual decrease in size from the periphery to the center (Fig. 2), but were practically the same size throughout, except for occasional very large ones (Fig. 2, *c*), which stained deeply with eosin. The spaces between the cells were filled with a loose mucoid material. The great bulk of the organ was formed of medium-sized cells, with clear nucleus and cytoplasm



that were comparable with the parietal cells of the active gland except for the granules in the cytoplasm (Figs. 1 and 2, *a*). The second kind of cells, far fewer in number, were much larger, with a small and indistinct nucleus and large amount of granular cytoplasm (Fig. 2, *c*). These cells were variously placed, some in the outer layers of the tubules, some in the mass of the cells, and some in what lumen there was to the tubes, for this part was greatly reduced and filled with loose tissue. The deep red stain that the large cells took distinguished them very readily from the rest, which showed more of the hæmatoxylin color. In shape and structure they suggested undeveloped or abortive *ova*, and on comparison with a section of ovary the similarity was striking, the chief difference being that the cells in the testis were smaller, had smaller nuclei and presented a degenerating appearance common to all the cells of the organ. There was no evidence of function in the gland, although the right testis was beyond doubt in an active condition.

In attempting an explanation of these facts, we touch the corner of one of the battlefields of science—the influence of environment on development. In embryonic growth the germ cells long remain comparatively undifferentiated; there is a certain anatomical position in the body that corresponds with certain stages of histological differentiation of the reproductive organs. In this case arrest occurred in an early anatomical position. Did histological differentiation cease soon after,

and did the abdominal position, normal to the ovary, so influence the cells of the organs as to lead to an attempt to develop ova after it had acquired distinct male characteristics? Did the ova come from any of the germ cells as they were then developed, or from some indifferent ones, which when subjected to conditions normal to the ovary began to develop into ova?

These are questions which arise and are difficult to answer, but suggest a possibility of the influence of environment on determining the line of development in an important matter and at a relatively late stage in embryonic life. I should be glad to know if any reader has observed a similar state of this organ in any animal, and if so what microscopic testimony it gives on this point. The tissues were hardened in four per cent. formalin with fifty per cent. alcohol, and gave good results both as to stain and preservation.

EDITH J. CLAYPOLE.

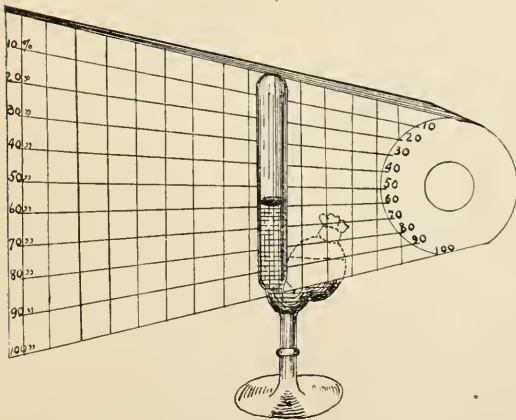
Wellesley College.

## A Simple Gasometer for Fermentation Tubes.

Since Theobald Smith's\* work on the fermentation tube, this piece of apparatus has become quite generally used and is a valuable agent in the study and differentiation of bacteria.

Where gas is formed it is desirable that the total amount of gas, as well as the relative proportion of the constituents, be determined. Graduations on the closed arm, as in the case of Einhorn's pattern, add to the expense more than is warranted by the degree of accuracy required, and therefore for bacteriological work

the tubes are ungraduated. The gas ratios are generally determined by measuring with an inch or centimeter rule and calculating the per cent. in terms of the length of the closed arm. Inasmuch as the tubes usually vary considerably in size, the calculations often become quite time-consuming. To overcome this, the piece of apparatus represented in the accompanying figure was devised, and has been used in these laboratories for some time.



It consists of a piece of triple-plated tin cut in the shape indicated, about eight inches long, six inches wide at one end and two inches at the other. The wide end is long enough to accommodate the largest fermentation tubes, while the narrow end is sufficiently short for the smallest. About a half inch of the

\* Wilder Quarter Century Book, p. 187.



upper edge is bent over, forming a right angle. The lines are ruled with common writing fluid and then a thin coat of shellac is brushed or sprayed over the entire surface. The perpendicular lines are about one centimeter apart, while those which run in the other direction are nine in number and are so drawn that they divide the perpendicular lines into ten equal segments. At each end of these lines the percentage is indicated by figures.

This apparatus is used in the following manner: it is grasped in the right hand, by the narrow end, while the fermentation tube is held in the left hand, bulb away from the body. The gasometer is then placed between the closed arm and the bulb, the bent edge resting on the top of the closed arm of the fermentation tube. The tube is now moved to the left until the neck, connecting the two arms, touches the lower edge of the tin, the perpendicular lines enabling one to hold the tube upright. In this position the amount of gas can be read off directly to within a per cent. or two, which is as accurate as the conditions warrant. The reading should be made from the center of the tube.

The bright tin surface acts as a mirror, and, in reading, the eye should be held in line with the meniscus and its reflection.

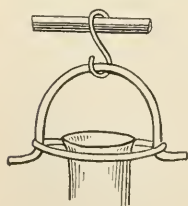
In working out the gas formula the amount of gas remaining after the treatment with caustic soda is noted; this represents the hydrogen, while the difference between this reading and the original (a subtraction which can be made mentally) is the carbon dioxide; e. g., let the total amount of gas be 60 per cent, the second reading (after treatment with Na OH) 40 per cent., then  $H : CO :: 40 : 20 = 2 : 1$ .

W. D. FROST.

Bacteriological Laboratories of the University of Wisconsin.

## Test-tube Suspenders for Cleaning Diatoms, etc.

In cleaning small quantities of diatoms, or other minute objects which require repeated washings in water to remove the acid, etc., it is important that the vessel in which the washings are performed—usually a test-tube—should

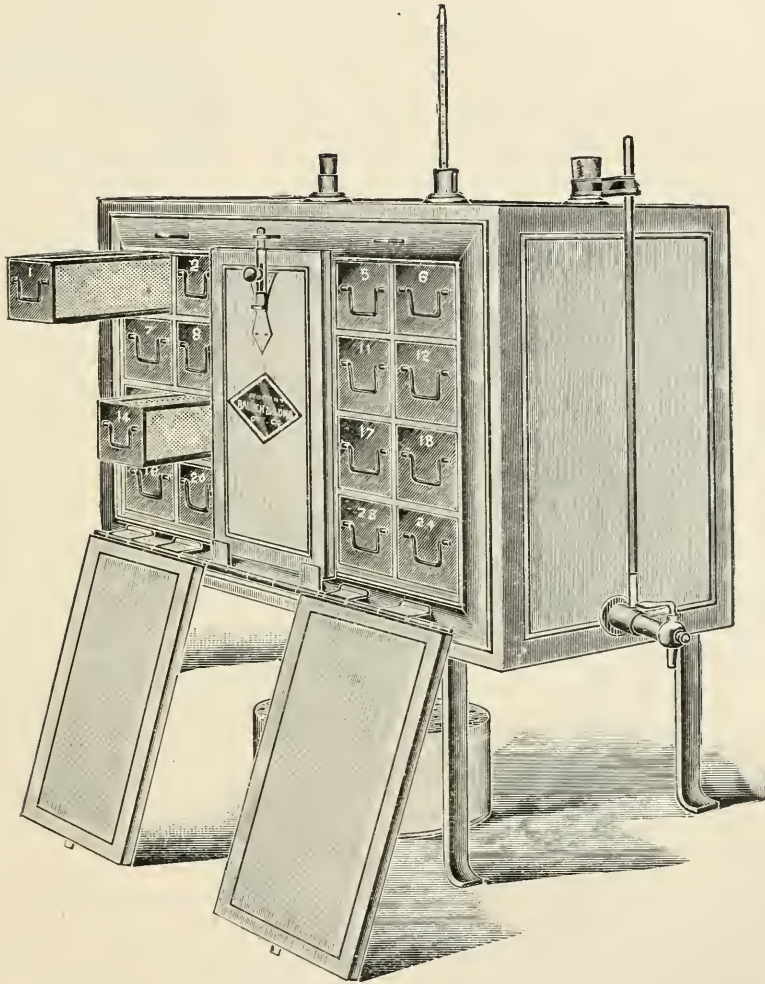


have its sides perfectly vertical, as otherwise the objects tend to rest against the sides and get carried off in the successive decantations. To ensure this, I find it a very convenient plan to suspend the test-tubes by a U-shaped piece of wire, across the base of which an india-rubber ring is fixed, enclosing the test-tube. The elasticity of the ring allows the tube to hang perfectly freely, and if it is set slightly swinging by air currents the precipitation of the

diatoms is rather improved, as they are prevented from lodging against the sides by the slight disturbance produced. The same device is often useful in the preparation of desmids and other small objects.

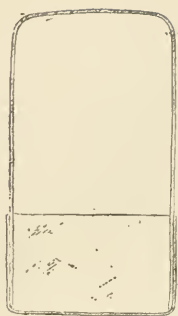
G. H. BRYAN.

## Dishes for Infiltrating Tissues in Paraffin.



THE LILLIE WATER BATH.

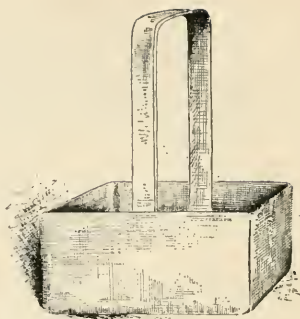
If one employs the Lillie oven for infiltrating tissues in paraffin, it is necessary to have small dishes to hold the paraffin. The little tin pattie dishes answer fairly well, but there is liability of spilling the paraffin, and also of getting the fingers uncomfortably hot. To overcome the difficulties, the dish shown half size in the accompanying figures was designed several months ago. It has answered the needs so admirably that it is published for the sake of other laboratories which may need such a dish to use with their ovens. It is made of tinned and burnished copper. The handle is of a strip of copper and



is on the inside of the box, as shown in the section. Of the size shown in the figure, one can easily put eight of the infiltrating dishes in a drawer of the large Lillie oven.

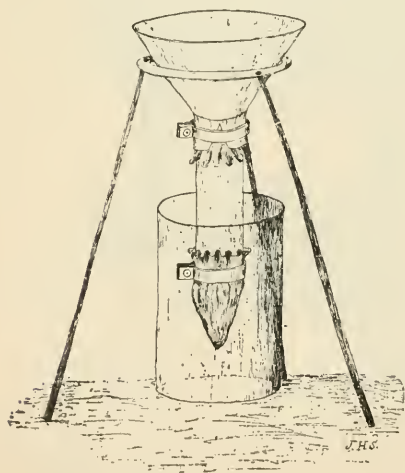
S. H. GAGE.

Histological Laboratory, Cornell University, Jan. 13, 1899.



## A Convenient Washing Apparatus.

The apparatus described below will be found convenient for washing material after being killed in an acid or other solution. It consists of a glass tube of suitable thickness, ten centimeters long and from two to three centimeters in diameter; two open brass rings with thumbscrews; a funnel of brass or tin about five centimeters wide at the top, four centimeters deep, and ending below in an open tube one centimeter long; and two cotton or linen cloths for strainers. The apparatus is put together as shown in the figure, and may be supported on a tripod.



When the objects are ready to be washed, remove the lower ring and cloth and pour the objects, with the solution in which they are contained, into the tube, and then replace the ring and cloth, and let the water flow into the funnel. Usually it will be found best to let the lower part dip into a glass dish, as shown in the figure. When the objects are washed they can easily be transferred to a bottle by taking off the cloth into which they will have settled. In this way small and delicate objects can be handled without injury.

This apparatus can also be used as a filter, or for collecting small water plants and animals. For instance, by having a cloth with a coarser mesh above and a finer one below, organisms of a certain size can be collected in the lower cloth free from foreign matter or larger animals and plants.

JOHN H. SCHAFFNER.

Botanical Laboratory, Ohio State University.

## LABORATORY METHODS IN BACTERIOLOGY.

DR. F. G. NOVY.

University of Michigan, Ann Arbor, Michigan.

## VI.—The Cultivation of Anaerobic Bacteria.

While the majority of bacteria live in the presence of air, there are some which are not favored by the free access of oxygen. It is customary therefore to designate as obligative anaerobic those bacteria which are obliged to live under anaerobic conditions—that is to say, where there is no air.

The exclusion of air from the culture medium can be accomplished, more or less satisfactorily, by a variety of methods. In so far as the principles of the methods employed are concerned, these may be spoken of under the following heads :

- |                          |  |
|--------------------------|--|
| 1. Exclusion of oxygen.  | 4. Displacement of air.                    |
| 2. Exhaustion of air.    | 5. Cultures apparently in presence of air. |
| 3. Absorption of oxygen. | 6. Microbic association.                   |

## EXCLUSION OF OXYGEN.

Several methods can be grouped under this head. Thus, the earliest method employed was to cover the liquid with a layer of oil. Although not perfect, yet this method of Pasteur can at times be employed to advantage. Later on, Koch endeavored to obtain colonies under anaerobic conditions by placing a thin *mica sheet* on the surface of a gelatin plate. Better results are obtained by covering the gelatin or agar plate with another sterile glass plate (Sanfelice).

The well-known method of Liborius, culture in *deep layers* of gelatin or agar, depends upon the exclusion of air. The method is simple and very convenient. The tube should contain glucose agar or gelatin, and the medium should be about two inches deep. Stab cultures are made in the usual manner. Growth develops along the line of inoculation in the lower two-thirds of the medium, while the upper third serves to exclude the air. In order to insure complete exclusion of oxygen, the contents of an ordinary agar or gelatin tube can be liquefied and then, with proper precautions against contamination, poured on top of the inoculated medium and quickly cooled. This extra layer, however, is not necessary if the medium has the depth given above.

Isolated colonies can be obtained by the method just given. The liquefied glucose agar or gelatin is inoculated in the usual manner as when making plates. The contents of the tube are then solidified and an extra layer of the medium can be poured on top.

The method of Roux deserves mention under this head. The inoculated medium is drawn up into a sterile glass tube pipette, which is then sealed at both ends by means of a flame.

## EXHAUSTION OF AIR.

Pasteur, in his studies on the bacillus of malignant œdema, employed special tubes which were connected with an air-pump, and when a vacuum was



reached they were sealed in a flame. The method has been simplified by Gruber and is easy of execution. Special test-tubes with a constriction below the cotton plug may be obtained. After the medium is inoculated a vacuum is produced in the tube, which is then sealed in the flame. If desired, colonies can be obtained by making a so-called "roll-tube culture" after sealing the tube.

#### ABSORPTION OF OXYGEN.

If a solution of pyrogallic acid is rendered alkaline, it will immediately become dark, then brown, and finally black, due to rapid absorption of oxygen. This reaction has been utilized in a variety of methods. Buchner's method consists in placing the inoculated tube inside of a larger one which contains on the bottom some pyrogallic acid. Caustic alkali is added to the acid, and the tube is then closed at once with a rubber stopper.

#### DISPLACEMENT OF AIR.

This can be accomplished by passing through the tube or apparatus a current of an inert gas. Hydrogen is usually employed for this purpose. The Liborius tube is to be mentioned in this connection. After the medium is inoculated a current of hydrogen is passed into the tube, through the side tube, until the air has been displaced. When this result is attained, the tube is sealed below the cotton plug, and finally the side-tube is likewise sealed. Various modifications of the original tube have been proposed, but although they render the method less expensive they do not materially simplify the procedure.

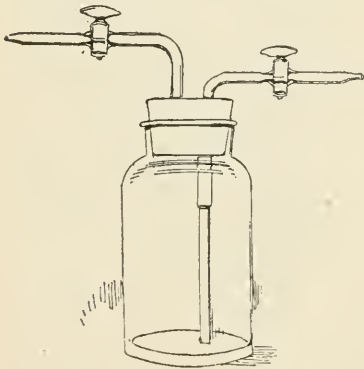


Fig. 1.

Fig. 1 shows an apparatus which was originally employed by the author. It can be constructed by any one, and is simple, inexpensive, and will give excellent results. Ordinary test-tubes are employed instead of the expensive tubes of Liborius. Moreover, a large number of tubes can be placed in one apparatus. The directions for use as given in connection with the author's special apparatus are applicable to this bottle. The stop-cocks serve to seal the apparatus.

A great variety of apparatus has been described for the purpose of obtaining colonies. Some of these, like Botkin's, enable one to make use of the ordinary Petri dishes. More often, however, they consist of a special dish such as that of Kitasato.

#### CULTURES IN THE PRESENCE OF AIR.

This method, from the nature of the organisms under consideration, would seem to be impossible. Nevertheless, as the author has shown, good cultures can be obtained without the use of any special apparatus, and these cultures are made apparently with free access of air. When a tube of glucose agar is liquefied and then allowed to solidify, a drop or two of water will separate out on the surface of the agar. If a stab-culture is now made, for instance of

malignant œdema, it will show a good growth not only along the line of inoculation in the deeper layers of the agar, but also in the liquid on the surface. This liquid is apparently in direct contact with the air. It is possible, however, that the carbonic acid and other gases given off by the organism displace the air from the tube and allow thus a growth to take place.

A better and more useful procedure than the above is to employ gelatin containing two per cent. of glucose and colored with litmus. The ordinary test-tubes containing this material are inoculated and placed direct in the incubator at 37°. Although the gelatin melts and apparently there is free access of air, yet abundant growths of all of the anaerobic bacteria can thus be obtained. The viscosity of the liquid undoubtedly prevents the access of air. It is evident that the author's method as just given is the simplest possible procedure. The cultures are readily accessible for examination, and, moreover, they retain their vitality longer than cultures on agar or in bouillon.

#### MICROBIC ASSOCIATION.

When a strongly aerobic germ, such as the *Micrococcus prodigiosus* or the *Proteus vulgaris*, is inoculated into a tube of bouillon, and if at the same time an anaerobic organism is planted, it will be found that both bacteria will develop. Apparently the aerobic form consumes the oxygen in the immediate neighborhood of the anaerobic organism and thus allows the latter to develop. Such cultures are intensely virulent. Conditions of this kind not only favor the growth of anaerobic bacteria in the soil, but are known to bring on disease. Tetanus or lockjaw is induced in this way as a result of mixed infection.

The various forms of apparatus indicated above, and described in most of the text-books, are far from being satisfactory. The use of special tubes or of special plates, where a large number are to be used, is a matter of considerable expense. The treatment of each tube by itself and the subsequent sealing involves a waste of time and is not altogether free from danger. It is obviously desirable to make use of the ordinary test-tubes and ordinary Petri dishes. This can be done by means of the author's special apparatus, shown in figures 2, 3 and 4. During the past six years this apparatus has been in constant use in the Hygienic Laboratory of the University of Michigan.

The bottle shown in Fig. 2 is intended for tube cultures. It is made in two sizes (8x16 and 10x 20 cm. inside) for large and for small tubes. It is provided with a hollow stopper, which should be not less than 4 cm. in diameter. There are openings in the glass stopper corresponding to the two tubes in the neck of the bottle. One of these openings has a glass tube attached which extends down to within a short distance of the bottom. After the current of hydrogen has passed through for some time, the stopper is turned at right angles, thus effectually sealing the bottle.

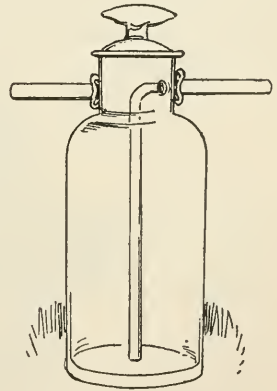


Fig. 2.

Ordinary test-tubes containing glucose bouillon, gelatin, agar, or potato, milk, etc., are inoculated in the usual manner. The projecting part of the cotton plug is cut off close to the mouth of the tube, and the plug is slightly raised, with sterilized forceps, to facilitate diffusion of the gas. The tubes are then placed in the bottle by means of a pair of rat or crucible forceps and the apparatus is connected with a Kipp's hydrogen generator. The hydrogen is generated from ordinary granulated zinc by means of commercial sulphuric acid. It is wholly unnecessary to employ chemically pure zinc and acid. The gas should be washed by passage through an alkaline solution of lead acetate and then through a six per cent. solution of potassium permanganate. A rapid current of hydrogen should be passed through the bottle for one to two hours, after which the stopper is turned at right angles. The bottle is then placed in the incubator.

When the cultures have developed they should be taken out of the bottle and preserved the same as ordinary bacteria. Care should be exercised when removing the stopper so as to avoid breakage. It should first be turned so as to allow air to enter. The thumb and forefinger of the left hand should rest firmly on the shoulder of the stopper while this is gradually worked to and fro with the right hand. This little precaution will prevent the sudden jerking out of the stopper.

The pyrogallate method can be used in connection with this bottle. Two to three grams of the acid can be placed on the bottom, the inoculated tubes introduced, and finally the necessary concentrated alkali (25 cc. 1:4) can be delivered from a pipette. The stopper must be inserted at once and turned at right angles.

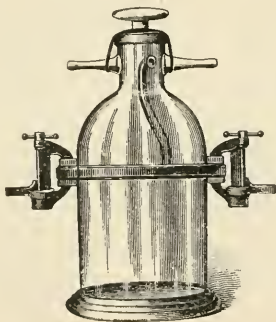


Fig. 3.

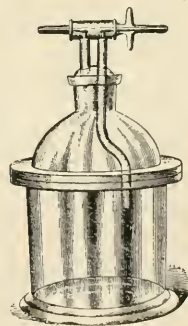


Fig. 4.

Figs. 3 and 4 show two forms of apparatus for obtaining plate cultures. The former is provided with a stopper like that in the bottle just described. This apparatus can be used for the gas or the pyrogallate method. The apparatus shown in Fig. 4 is provided with a slightly different stopper, and can be used for the gas, pyrogallate, or vacuum method.

The lower half of the apparatus (Figs. 3 and 4) should have an internal diameter of 12 cm. and an inside height of 12 cm. The apparatus can hold six or eight Petri dishes. It can be used for small flasks or for a large number of tube cultures. The total height of either apparatus should not exceed 24 cm. Each flange should be 2 cm. wide and 3-4 cm. thick. The outer circumference

should be ground vertically and the top and bottom should have exactly the same diameter. A rubber band can then be slipped over the circumference of the two flanges, thus materially assisting in closing the apparatus. The unground surface of the flanges should be parallel or nearly so in order that the clamps will not slip off.

The gelatin or agar Petri dishes are placed in the lower jar. It is unnecessary and undesirable to remove the tops. The ground surfaces of the apparatus should be lubricated with a mixture of beeswax and olive oil (1:4). The upper part is then placed in position and the wide rubber band is slipped over the circumference. Three clamps (No. 1 amateur vise, Phoenix Hardware Co., Phoenix, N. Y.) are applied to the flanges. A slit piece of rubber tubing should be slipped over the jaws of the clamps. Hydrogen is then passed through the apparatus in the manner described above. Finally the stopper is turned and the apparatus can then be set aside for the organisms to develop.

In this way colonies of the anaerobic bacteria can be obtained with the greatest ease, and what is important, they are accessible for observation and transplantation. The pyrogallate method can be used with equally good results. Three to five grains of pyrogallic acid are placed in a dish which is about 10 cm. in diameter and 3 cm. high. This is placed on the bottom of the lower jar and covered with a strip of glass about 5 cm. wide. The Petri dishes are then stacked on top. The upper half of the apparatus is placed in a position so that only a narrow slit remains open. By means of a pipette or other arrangement 25 cc. of concentrated potash solution (1:4) are added as rapidly as possible. The top is then closed completely and the rubber band and clamps applied as before.

Hygienic Laboratory, University of Michigan.

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A Biological Society has been organized at De Pauw University, with a limited membership of twenty-five composed of advanced students of the university. The membership in this society is based on merit of students. Prof. Mel. T. Cook will soon publish a thesis on the water lily.

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H. H. Z.

At a recent meeting of the board of managers of the New York Botanical Garden, Dr. D. T. MacDougal, professor of physiology in the University of Minnesota, was appointed to fill the newly created position of director of the laboratories. Dr. MacDougal has accepted the appointment, and has handed his resignation to the president of the University of Minnesota, to take effect July 1st, when he will assume his new duties in New York.



# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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Issued Monthly from the Publication Department  
of the Bausch & Lomb Optical Co.,  
Rochester, N. Y.

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DR. R. M. PEARCE, Pathological  
Laboratory, Boston City Hospital, will  
conduct a department of Pathological  
and Normal Histology along the lines  
of the departments of Current Botani-  
cal Literature, Animal Biology, and  
Current Bacteriological Literature, be-  
ginning in the April number of the  
JOURNAL. The object of this depart-  
ment is to put our readers in touch  
with the best writings on these sub-  
jects in all languages, translated into  
English, and with complete references.

\* \* \*

It has been aptly said that the suc-  
cessful educator of to-day is, primarily,

a business man. The most successful educational institutions, and the scientific laboratories in which the student receives the greatest benefit, are those conducted on business principles. In the higher educational institutions, the management of the laboratory depends almost entirely upon the individual in charge of that department, and it is reasonable to expect that competition may be depended upon to introduce the most desirable conditions. In the public schools the conditions are altogether different, owing to the fact that, while the management of the laboratory and its equipment depends just as fully upon the individual as in the college or university, the majority of science teachers in the secondary schools hold their positions only a comparatively short time, and they are as a rule comparatively immature as to teaching experience, certainly so as to the application of business methods to the conduct of the laboratory. The desirability of a change in the direction of better business methods in the laboratories of these schools is evidenced in the lack of uniformity in the results of teaching in various schools in the same state, the unwise expenditure of the funds of the school by persons who are uninformed as to what apparatus may be had for any particular purpose, much less the best and most economical, and to a certain extent the unconscious, perhaps, hobby riding of the various instructors, one having a certain interest in electricity, spending the larger part of the equipment fund for electrical apparatus, and so on through the list. The State of New York has demonstrated in a practical way that these defects may be remedied to a large extent by a rational state supervision. This supervision has resulted in the adoption of a uniform course of study throughout the state, the uniform equipment of schools with apparatus suited for the illustration of the subjects in the required course, and the saving of thousands of dollars to the schools through information furnished by the state regarding the value of books, apparatus, and materials required. The equipment provided is kept up to the standard by inspectors who visit each school, and some of whom are mechanical experts, capable of repairing or directing the repair of any damaged or worn apparatus. A movement along this line is on foot in other states, and will no doubt be aided by all interested in the advancement of the public schools.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Nawaschin, S.** Neue Beobachtungen über Befruchtung bei *Fritillaria tenella* und *Lilium Martagon*. Bot. centralbl. **77**: 62, 1899.

fertilization in *Fritillaria* and *Lilium*. He claims that in the development of the egg apparatus a genuine cellulose membrane is formed around the synergids and the egg, but that this membrane is resorbed before the entrance of the male sex cells into the embryo-sac. He thinks that the male cells are motile. Both male cells enter the embryo-sac, one of them then pressing into the egg while the other fuses with the nearest polar nucleus. This fusion nucleus then unites with the other polar nucleus, so that the primary endosperm nucleus is formed by the fusion of three nuclei. It is hard to see any significance in such a fusion, and considerable confirmatory evidence would be needed to support the observations.

**Davis, Bradley Moore.** Kerntheilung in der Sporenmutterzellen bei *Corallina officinalis* L. var. *mediterranea*. Ber. d. deutsch. bot. Gesell. **16**: 266-272, 1898.

tetraspore mother cell is yet in the resting condition, it is surrounded by a layer of denser cytoplasm, but no centrospheres are recognizable. Previous to division the nucleus elongates; meanwhile there appears at each of its ends a considerable aggregation of thick protoplasm, which becomes more and more dense until it may be resolved into a centrosphere and a surrounding mass of kinoplasm. In a later stage the latter is transformed into a collection of radiating threads. The centrosphere when fully differentiated is a quite large homogeneous body, a distinct centrosome being at no time present. When division is completed the centrospheres and astral radiations are gradually transformed again into an irregular kinoplasmic mass. The author accordingly concludes that in *Corallina* the centrosphere is not a permanent cell-organ, but arises *de novo* from the cytoplasm in the early phases of karyokinesis. Another interesting feature is the apparent want of a lining network. Hence the chromatin appears in the resting nucleus in the form of irregularly scattered granules. In the formation of the daughter nuclei the chromatin is fused into a globular mass, distinguishable from the nucleolus only by suitable staining. In older nuclei the chromatinsphere breaks up into the scattered granules already mentioned.

WILSON R. SMITH, Chicago.

**Campbell, D. H.** On the structure and development of *Dendroceros*, Nees. Linn. Journ. Botany. **33**: 467-478. pl. 21 and 22, 1898.

two genera, occurring in temperate zones and so within easy reach of botanists,

At a recent botanical congress in Kiew, Russia, Prof. Nawaschin made some rather sensational remarks concerning

He claims that in the development of the egg apparatus a genuine cellulose membrane is formed around the synergids and the egg, but that this membrane is resorbed before the entrance of the male sex cells into the embryo-sac. He thinks that the male cells are motile. Both male cells enter the embryo-sac, one of them then pressing into the egg while the other fuses with the nearest polar nucleus. This fusion nucleus then unites with the other polar nucleus, so that the primary endosperm nucleus is formed by the fusion of three nuclei. It is hard to see any significance in such a fusion, and considerable confirmatory evidence would be needed to support the observations.

C. J. C.

This paper is of special interest as a record of the first successful investigation of the karyology of one of the Florideæ. When the nucleus of the

it is surrounded by a layer of denser cytoplasm, but no centrospheres are recognizable. Previous to division the nucleus elongates; meanwhile there appears at each of its ends a considerable aggregation of thick protoplasm, which becomes more and more dense until it may be resolved into a centrosphere and a surrounding mass of kinoplasm. In a later stage the latter is transformed into a collection of radiating threads. The centrosphere when fully differentiated is a quite large homogeneous body, a distinct centrosome being at no time present. When division is completed the centrospheres and astral radiations are gradually transformed again into an irregular kinoplasmic mass. The author accordingly concludes that in *Corallina* the centrosphere is not a permanent cell-organ, but arises *de novo* from the cytoplasm in the early phases of karyokinesis. Another interesting feature is the apparent want of a lining network. Hence the chromatin appears in the resting nucleus in the form of irregularly scattered granules. In the formation of the daughter nuclei the chromatin is fused into a globular mass, distinguishable from the nucleolus only by suitable staining. In older nuclei the chromatinsphere breaks up into the scattered granules already mentioned.

WILSON R. SMITH, Chicago.

The Anthocerotaceæ, as is well known, consist of three genera, *Anthoceros*, *Notothylas*, and *Dendroceros*. The first

The first two genera, occurring in temperate zones and so within easy reach of botanists,

have been frequently studied, but *Dendroceros*, being exclusively tropical, has been necessarily neglected, the only important account being that of Lietgeb, who had only herbarium material to work with, and even this consisting mostly of mature plants. While visiting Jamaica in the summer of 1897, Prof. Campbell put up an abundant supply of material in chromic acid and in alcohol. The plant is an epiphyte, growing upon twigs and stems of small trees and shrubs, and also upon the upper surface of the leaves. A part of the summary is about as follows: in its apical growth and form of the thallus, *Dendroceros* differs decidedly from the other genera of the order. The type of apical cell is that found in *Pellia* and in the homasporous ferns, especially *Osmunda*. The abnormally large chloroplasts of *O. cinnamomea* may indicate a relationship with forms like *Dendroceros*. The archegonium corresponds in structure with those of *Notothylas* and *Anthoceros*, and is intermediate in character between these. The antheridium is solitary and is endogenous in origin. The first wall in the embryo is longitudinal, as in *Anthoceros*, but the first transverse wall determines the limit of the foot, as in *Notothylas*. The archesporium comes from the amphithecium as in the other two genera. The division of archesporial cells into sporogenous and sterile ones is less regular than in the other genera, and primary archesporial cells may become sporogenous ones without further divisions. The elaters have a thickened spiral band. In *D. crispus* (?) the spores germinate within the capsule. No stomata were found on the capsule. *Dendroceros* shows a close relation to *Anthoceros*. In the form of the archegonium and in the early stages of the embryo, *Dendroceros* is intermediate between *Notothylas* and *Anthoceros*. Two excellent plates illustrate the development of the thallus, archegonium, antheridium, and sporophyte. C. J. C.

**Campbell, D. H.** Recent work upon the development of the Archegonium. Bot. Gaz. 26: 428-431, 1898.

Prof. Campbell takes exception to Gayet's work on the development of the archegonium in the liverworts and mosses. (Gayet's work appeared in the Ann. d. Sci. Nat. Bot. VIII. 3: 161-258, 1897, and was reviewed in the Bot. Gaz. 25: 68-69, 1898.) The prevalent opinion is that in mosses the growth of the archegonium is largely apical, while in liverworts it is for the most part intercalary, the early division of the "cover cell" in liverworts preventing additions to the neck from this source. Gayet claims that the terminal cell, often called the cover cell, contributes to the growth of the neck of the archegonium in liverworts as well as in mosses. Gayet also claims that neck canal cells in mosses are not cut off from the base of an apical cell, as is generally supposed, but have the same origin as in liverworts. This means that he regards the type of archegonium as essentially the same in liverworts and mosses.

In his book on Mosses and Ferns, Prof. Campbell showed that there is an essential difference between the development of the liverwort archegonium and that of the moss, the cover cell of the liverworts very early undergoing the quadrant division and thus becoming incapable of functioning as an apical cell, while in the moss the apical cell persists for a long time and contributes both to neck cells and the neck canal cells. In regard to the liverworts the present paper gives convincing evidence in favor of the view already expressed by the

writer in his book. Gayet's paper gives unmistakable proof that his technique was defective. He does not seem to have used the paraffine method at all, and his stains were not suited to bringing out cell walls. His primitive methods and his failure to read the book on Mosses and Ferns make Gayet's results rather questionable where they differ from those of Prof. Campbell.

C. J. C.

**Jordan, E. O.** The production of fluorescent pigment by bacteria. *Bot. Gaz.* **27**: 19-34, 1899.

In this publication there are presented some very interesting results bearing upon the production of fluorescence

by bacteria.\* In the experiment six cultures representing as many so-called "species," were grown in various nutrient solutions of chemical compounds, the molecular composition and arrangement of which were accurately known. When in these solutions no sulphates were present, no fluorescence could be obtained; but when sulphates were present in sufficient quantity, fluorescence appeared. The quantity of sulphate required is extremely small, 0.00001 per cent. in the case of one of the cultures used being sufficient, while 0.0001 per cent. sulphate resulted in the development of good fluorescence by five of the six species. It seems quite likely that those who have reported fluorescence in the absence of sulphate have used commercially prepared nutrient solutions, which frequently contain sulphates, although they may be marked "C. P." This was probably true with Thumm's experiment.

The base associated with the sulphate does not seem materially to affect the production of pigment. In greatly concentrated solutions of nutrient substances fluorescence is not so bright nor so persistent as in simpler solutions. Cultures kept in the dark fluoresce more readily than those constantly in diffuse light. The presence of an acid serves not only to neutralize the pigment already produced, but inhibits further development of pigment. The production of these pigments is probably not worked out in order to assist the bacteria in obtaining proper nourishment, but rather they probably represent some of the products which accompany the metabolic processes of these organisms. When certain nutrient substances are present these pigments may be so formed. When other nutrient media are present, metabolic by-products are formed which do not chance to be fluorescent.

OTIS W. CALDWELL, Chicago.

**Nelson, B. E.** Microscopical examination of tea, coffee, spices, and condiments. (Chemical Laboratory, Binghamton, N. Y. State Hospital.) Reprint from Merck's Report, 1898.

Since the trade in spices and condiments has been drifting away from the druggist to the grocer, adulteration has been constantly increasing. The

determination of the purity of these articles by chemical analysis is tedious and often unsatisfactory, but the microscope furnishes a ready means of examination. It is necessary, in the first place, to become familiar with the histological characters of teas, coffees, spices, etc. This paper gives numerous figures illustrating histological characters, together with descriptions and directions for preparing the material for microscopic examination. The articles figured and described are tea, coffee, pepper, capsicum, caraway, ginger, mustard, cassia buds, cinnamon, cloves, allspice, mace, and nutmeg.

C. J. C.

\*Beiträge zur Biologie der fluoreszierenden Bakterien. *Arb. d. Bakteriolog. Inst. d. grossh. Hochschule zu Karlsruhe*, 1895.



**Caldwell, O. W.** On the life history of *Lemna minor*. Bot. Gaz. **27**: 37-66, figs. 1-57, 1899. The sporophyte of the Lemnas, so abundant in ponds and sluggish streams, has been frequently investigated, its greatly reduced character making it of special interest. The plants are seldom found in flower and the gametophytes have never been studied in detail. In August, 1897, Dr. Caldwell found an abundance of *Lemna minor* in flower. As far as the structure of the sporophyte is concerned, the writer's observations agree in general with those of previous investigators, but he draws the conclusion that "the sporophyte of *Lemna minor* cannot be definitely homologized with either a stem or a leaf, but is a shoot undifferentiated except at the basal and nodal regions." The development of the microsporangia and macrosporangia, and particularly the gametophytes, is traced in detail. In each stamen there appears a single archesporial mass, which becomes divided into four masses, or the four loculi of the anther, recalling the condition of affairs in *Isoetes*. The four loculi would then constitute one sporangium and not four. If this interpretation is correct, it will doubtless apply to many other plants. The cells of the tapetum frequently divide and push into the loculus. Many microspore mother cells disintegrate and function as tapetal cells. The generative cell divides into the two male cells before the pollen grain is shed.

In the macrospore series the archesporial cell gives rise to a tapetal cell and a sporogenous cell, the latter developing directly into the megaspore. It is interesting to note that embryo-sac structures rarely reach a complete normal development, disorganization usually checking the process at some stage. It follows naturally that embryos are extremely infrequent. The numerous excellent illustrations are scattered through the text instead of being grouped into plates in the usual manner. In some respects this is very convenient, but it deprives one of the birds-eye view furnished by a well arranged plate, and when illustrations are drawn to the same scale one loses the impression of comparative size. It remains to be seen whether this feature will find favor with investigators.

C. J. C.

**Schwendener, S.** Gesammelte botanische Mittheilungen. 8vo. **1**: pp. VIII + 453. figs. 15, pl. 11. Vol. **II**. pp. VI + 419. figs. 8, pl. 15. Berlin: Gebriüder Bornträger, 1898. M25.

It is very fortunate for botanists that the numerous papers published by Prof. Schwendener during the past twenty years, in the *Berichte* and

*Abhandlungen* of the Royal Prussian Academy of Sciences, and other places, have finally been brought together in convenient form. The papers are arranged according to subject and the contributions on each subject are arranged in chronological order. The subjects treated in the first volume are: trajectory curves; stomata; phyllotaxy; ascent of sap; swelling and double refraction; twining of plants. In the second volume the subjects are: stability of plants; growth; latex vessels; protective sheaths; pulvini. The volume closes with two papers by Schwendener and Krabbe.

**Stevens, Wm. C.** Ueber Chromosomentheilung bei der Sporenbildung der Farne. Ber. d. deutsch. bot. Gesell. **16**: 261-265. pl. 15, 1898.

Most botanists believe that a reduction division of the chromosomes (in Weismann's sense) does not occur in plants.

Guignard, Strasburger, Sargant, Mottier, and many others have found that there

is always a longitudinal division of the chromosomes and consequently only a numerical reduction. Schaffner finds a transverse division and consequently a qualitative division (reduction division) in the first division of the nucleus in the embryo-sac of *Lilium*. Belajeff believes that a reduction division occurs in the second division of pollen mother cells. Calkins reports a reduction division in ferns. Mr. Stevens has studied this problem in the ferns, *Scolopendrium vulgare*, *Cystopteris fragilis*, and *Pteris aquilina*. A weak solution of Flemming's mixture is recommended for fixing. It may be used hot or cold. Chloroform was used to precede the paraffin. The safranin, gentian violet, orange combination gave the most satisfactory staining. The summary is about as follows: in the first division of the spore mother cell, the spireme thread splits longitudinally and then segments into one half the usual number of chromosomes. The daughter chromosomes are short and thick and in their form resemble tetrads, but there is no transverse division. The daughter chromosomes begin to separate, sometimes at the end and sometimes in the middle, thus forming double rods or ring-shaped chromosomes. In the daughter nuclei, the chromosomes fuse into a single nuclear thread. In the second division this thread splits longitudinally, and then segments transversely as in the first division. It follows that there is merely a reduction in the number of chromosomes and not a reduction division. The writer thinks that Calkins' figures lack important stages and do not prove a reduction division. No centrosomes or multipolar spindles were found.

C. J. C.

**Polariscope and Bacterial Cultures.** Fuenfstueck  
Beiträge z. Wissensch. Bot., **3**: 1-176, pl. 1-39.  
f. 1-2.

cultures of typhoid, diphtheria, tuberculosis, cholera, and *Bacterium monanchae*. Supposedly pure cultures of those gave way to other predominating forms after longer or shorter periods, with reduction in size of some species. A polariscope test in ordinary gelatin tubes, with mixed cultures obtained from water, revealed certain characteristic differences. Of 175 zoöglæ examined, 115 did not change the optical relations on the surface. Forty-seven had the axis of greatest elasticity tangential, 13 radial.

N. J. C. Muller, in a long contribution on certain pathogenic bacteria, discusses the question of morphology and impure

L. H. PAMMEL.

## RECENT LITERATURE.

**Ball, C. R.** Seed testing: its importance, history and some results, with a partial bibliography. Contributions from the bot. dept. of the Iowa State College of Agriculture and Mechanic Arts. No. 9, 1898.

**Chamberlain, C. J.** The Homology of the Blepharoplast. Bot. Gaz. **26**: 431-435, 1898.

**Eriksson, Jakob.** Etude sur le *Puccinia ribis* DC. des groseilliers rouges. Revue gen. d. Bot. **10**: 497-506, 1898.

**Hammerle, J.** Zur physiologischen Anatomie von *Polygonum cuspidatum*. (Inaug. Dissertation.) Göttingen, 1898.

**Hecke, L.** Untersuchungen über *Phytophthora infestans* als Ursache der Kartoffelkrankheit. Berlin, Jour. f. Landwirthsch. 1898.

**Nordhausen, M.** Beiträge zur Biologie parasitärer Pilze. Jahrb. f. wiss. Bot. **33**: 1-46, 1898.

**Rewitz, B.** Untersuchungen über Zelltheilung II. Archiv f. mic. Anat. u. Entwicklungsgeschichte. **53**:—, No. 1, 1898.

**Vejdovsky, F. and Mrazek, A.** Centrosom und Periplast, Vorläufige Mittheilung. (Separate from the Sitzungsber. d. Königl. böhmischen Gesell. 11 p. 6 figs., 1898.)

**Westermaier, Max.** Historische Bemerkungen zur Lehre von der Bedeutung der Antipoden-Zellen. Ber. d. deutsch. bot. Gesell. **16**: 214-216, 1898.

**Ziegler, H. E.** Experimentelle Studien über Zelltheilung. Archiv f. Entwicklungs-Mechanik der Organismen. **7**: No. 1. 2 pl. and 12 figs., 1898.

## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to

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## CURRENT LITERATURE.

**Apathy, Stefan.** Die leitende Elemente der Nerven Systems. Mitteilungen aus der Zoologischen Station zu Neapel, **12**: 4.

Gold chloride has come to be considered as of especial value in studying the finer points of neurological anat-

omy. As all the possibilities of this reagent have not yet been determined, any new details regarding its use are of interest. The Mitteilungen aus der Zoologischen Station zu Neapel, gives the method as applied by Dr. Stefan Apathy in his study of the relations between nerve fibers and nerve cells. Apathy was fourteen years in perfecting the method, which, with the characteristic altruism of the scientist, he gives to the public.

The salt used was aurum chloratum flavum ( $\text{AuCl}_3 \cdot \text{H}_2\text{O} + 4\text{H}_2\text{O}?$ ) in preference to aurum chloratum fuscum ( $\text{AuCl}_3 + 2\text{H}_2\text{O}?$ ), and there are two methods of application, one in which the gold chloride is used on fresh tissue, and another in which the material is first fixed in some killing reagent. The two methods give opposite results. In the first case the cytoplasm is stained, but the nucleus is left almost colorless, primitive contractile fibers are very pale, and interfibrillar substance is almost as dark as the cytoplasm of the muscle cell. The whole nerve, as distinguished from the myelin sheath, is more or less dark reddish-violet, so that the axis cylinder of the vertebrate nerve, for example, would be clearly differentiated, and the peripheral branchings could be followed. Gold chloride applied after fixation gives a very clear nuclear stain, the chromatic as well as the achromatic elements being affected, so that in karyokinetic figures the chromosomes, the spindle fibers, and the centrosome are stained with different intensities, the chromosomes and the nucleolus of resting nuclei being most deeply colored. The cytoplasm is paler, but plasmic structures are sharply stained. Thus the plasma of the muscle cell is pale, but the contractile primitive fibrils are bright cherry seal.

Nerve fibers are stained intensely black. Inter and peri-fibrillar substances take a very light stain, which differs from the other so that the neural substance is very clearly differentiated. Gold chloride may be used equally well on tissue from marine, fresh water, or land animals.

In the first method, the fresh material is placed in the dark in a one per cent. solution of gold chloride crystals in distilled water, for at least two hours, or, in the case of thin membranes, over night. Then without washing it is changed to a one per cent. solution of formic acid and exposed to the light in such a way that the rays of light may reach it on all sides, and left for at least six or eight hours. After the first hour, when the fluid has become dark, and therefore absorbs considerable light, the formic acid should be renewed, care being taken to move the object as little as possible. When the reaction is completed, the

washing out of the acid is not necessary, although it will do no harm. The object may be mounted directly in gum syrup or in concentrated glycerin. There is danger of shrinking the material in the dehydrating and clearing necessary for mounting in balsam. The characteristic reaction may be obtained in material that has been dead for some time, or after maceration in a one-third solution of alcohol for a day. Fine preparations, showing the end branchings of the nerve in isolated plates of the electric organ of Torpedo, may be obtained in this way.

The second method: Invertebrate tissue is fixed in a concentrated solution of sublimate in one-half per cent. sodium chloride; or in sublimate alcohol which consists of equal parts of the above solution and absolute alcohol. If it is specially desired to show the outline of the cell, a fluid consisting of equal parts of the sublimate solution and one per cent. osmium tetra-oxyd may be used. This is also preferable for vertebrate tissue. Large pieces of tissue are left in the sublimate alcohol from sixteen to twenty-four hours, thin membranes from four to five hours, or half that time in sublimate. The osmium solution is used like the others except that it requires washing in running water for six hours, and the steps preliminary to imbedding should involve the least possible exposure to light. After fixing, the object is washed in a solution of one per cent. KI and one-half per cent. I in water. Wash for from six to eight hours, frequently renewing the liquid, then bring the material directly into 95 per cent. alcohol, and leave over night; change to a solution of one per cent. KI and one-half per cent. I in 95 per cent. alcohol, until it is thoroughly yellowed, when the iodine is washed out with absolute alcohol. Objects thin enough to be transparent without sectioning are not placed in alcohol, but washed in the iodine solution. The quicker the material can be imbedded after fixing, the more successful the action of the gold chloride will be. Chloroform, or a solution of four parts of chloroform and one part ether, should be used in preference to xylol for clearing. Imbed in paraffin or celloidin. From seven to ten micra is the best thickness for sections. Fix the paraffin sections to the slide with distilled water, or with albumen fixative, the celloidin sections by the bergamot oil method. Remove the paraffin with chloroform, and bring the sections, by the usual steps, to distilled water, where they are left for at least two or, at most, six hours. Or, they may be rinsed in distilled water, then placed in a one per cent. solution of formic acid for a moment, and rinsed again in water.

The slide is then placed in a tube of one per cent. gold chloride for twenty-four hours, or at least over night. Dip the slide quickly in distilled water or remove the superfluous gold chloride from the glass (not from the sections) with filter paper, and immerse the slide in a glass tube of one per cent. formic acid, placed where it will receive the maximum of light on all sides, with the minimum of heat. The slide should be placed obliquely with the sections on the under side, where they will be protected from the precipitate which forms. After twenty four hours rinse in distilled water and mount in the usual way in balsam. or directly in concentrated glycerin or gum syrup.

For either method, the most important condition is that the light rays shall reach the tissue on all sides, and penetrate it with the greatest possible intensity



but without allowing the temperature to rise above 20° C. The sum of the light, heat, and chemical energy should form a constant which produces the desired changes in the gold solution. Diminution of one form of energy may be compensated by increase of another, although strong daylight is an essential. The reaction may be divided into two parts, the penetration of the gold chloride, and the action of the light upon the tissue. The tissue becomes opaque under the action of the reagent, consequently it must be a membrane, or consist of fine fibers, or be cut in thin pieces. As a rule objects are exposed to the light in the acid for at least eight hours in cool weather, and six hours in warm weather. Only occasionally in winter are objects left longer than twenty-four hours in the acid. The gold chloride may be left in the light as long as there is no organic matter in it. Organic matter causes a precipitate of metallic gold which weakens the solution. Long objects immersed in the fluid must be fastened at both ends, and membranes must be expanded and fastened, preferably, to glass rings which will allow free action of the light. The membranes should not be stretched too tightly. The reaction is accelerated by the access of the oxygen of the air. On this account the tissue may be floated on the surface of the liquid during reduction, or it may be placed in moist chamber, where the acid may be reduced from time to time.

E. M. B.

**Buchler, Dr. A.** Structure of Nerve Cells.  
Verh. Phys. Med. Ges. Wuerzburg, **31**: 1898,  
pp. 285-392.

The author gives a minute description of the spinal ganglion cells of a variety of types. A granular meshwork is present, in the interstices of which it is easy for fluid to circulate, and food can come and waste go out. The framework is associated with central corpuscles, and fibrils linking one ganglion cell to others are differentiations of this cell substance as paths for stimuli. The fibrils spread out in the cell are ultimately connected with the cell as a whole, both mechanically and physiologically. Each element of the cell is influenced by the others, and nervous function is the result of their united coöperation.

This author does not speak of any differentiation into two kinds of systems, such as Arnold shows in his work, but both pieces of work help to show the complexity of the protoplasmic structure in cells.

A. M. C.

**Kolossow, Prof. A.** Intracellular Connections in Epithelium. Archiv. Mikr. Anat. **52**: 1898, pp. 1-13.

In this work evidence is brought to show that epithelial cells are organically connected, instead of being merely cemented, as is usually supposed to be the case in epithelial tissue. The bridging is a constant structure and suggests the demonstration is not possible, that extra cellular bridges are of importance in glands and epithelium generally, especially as paths for the passage of stimuli from cell to cell, as well as useful for merely mechanical purposes.

A. M. C.

**Assheton, R.** Development of the Pig during the first ten days. Quart. Journ. of Micro. Sci. New Series No. 166, Nov. 1898, pp. 329-361, 4 plates.

In work done previously to this, no observations have been made on specimens younger than twelve to fourteen days; the author succeeded in getting a number varying in age from four days to eleven days, covering the earliest

stages. Ova at these ages were in almost all cases already in the folds of the uterine wall, and the method of procedure in obtaining and preserving material was as follows:

Shortly after the death of the animal, three-fourths of an hour to three hours, the upper end of the uterus was filled as completely as possible with .25 or .5 per cent. chromic acid solution, the injection being carried as far as possible to stretch the folds of the lining mucous membrane of the organ, and so float the ova free into the liquid. After ligaturing the lower end, the whole preparation was put in .5 per cent. chromic acid for two or more days. Later, the contents of the uterus were allowed to flow out into a glass and the ova found by careful search with the microscope. Chromic acid was found most satisfactory, owing to its clearness and the absence of any precipitate. Flemming's solution was used in some cases with equal success, but Perenyi or nitrate of silver caused too much precipitate and prevented the finding of ova. All the specimens were stained in toto in either hæmalum, carmalum, hæmatoxylin, or borax carmine, and imbedded in paraffin by the cedar oil method.

About one hundred embryos were obtained, thirteen of which were less than five-celled and twelve more preblastodermic. The most noteworthy point in connection with cleavage is that after the two-celled stage the blastomeres become markedly unequal in size, especially in from eight to twelve-celled stages. No difference is marked beyond that of size and the smaller segments are arranged as a cap on the larger; the inequality is more marked than in other mammals so far studied.

The cavity of the blastodermic vesicle is formed by vacuolation of the inner larger cells of the morula, not by the formation of an intercellular cavity. This makes the development of the pig more closely resemble that of the rat, according to Robinson, in this particular point. As evidence of this process, is given the intense vacuolization of the cells at this stage, the existence of strands of protoplasm reaching across the cavity after its formation and the position of oil globules such as are in the cells, free in the cavity. The end of this stage is marked by the rupture of the zona radiata. This latter event is attended by a great change in the form of the embryo, owing to relief from pressures. The hitherto spherical embryo becomes collapsed and irregular in outline and remains so till a considerably later stage. It is now a vesicle whose walls are one cell thick, forming a layer known as the trophoblast.

At one point on the inner wall of the trophoblast is a small mass of cells that is the future epiblast of the embryo.

During the eighth and ninth days changes are not remarkable, but already the hypoblast is recognized as separating from the epiblast, both layers being enclosed in a vesicle of trophoblast. On the tenth and eleventh days growth of the inner layers results in a rupture of the trophoblast, and the epiblast comes to the surface. In later stages masses of cells are found adhering to the outside of the blastodermic vesicle, which are considered the remains of the trophoblast. These cells do not necessarily die on rupture of the vesicle, but may persist for some time and even multiply. There is no evidence that they ever fuse with the epiblast.

The author uses Hubrecht's terminology, but does not accept his conclusions regarding the trophoblast. Assheton considers that early outer layer as hypoblastic in origin. The irregularity of the cleavage, the origin of the epiblast and hypoblast and relations to the trophoblast, and the fate of the latter form the most important points in this paper.

A. M. C.

**Morpurgo, B.** Über die postembryonale Entwicklung der quergestreiften Muskeln von weissen Ratten. *Anat. Anz.* **15**, pp. 200-206.

The author's investigations were carried on to determine the fundamental question whether muscles arise directly from fibers present at birth, or whether a new formation takes place. It has recently been maintained that after birth the actual number of fibers decreases. White rats were chosen for the studies and specimens were taken at given intervals, newly born, fifteen days, one month old, and full grown; care being taken to have animals of the same family. The author finds that in the first period of growth, up to fifteen days, an actual increase in the number of fibers takes place; new structures rising by the mitotic division of generalized elements present in the tissue. After the conclusion of these processes no further increase in the number of fibers takes place, but with increasing length in the fibers the nuclei multiply amitotically. The actual thickening of muscle fibres takes place independently of increase in number of fibers or nuclei; it has direct reference to the amount of contractile substance. The three different steps are distinct in significance. The first feature, number of fibers, is determined by the inheritance and fundamental plan of the animal. The amitotic division, the second step, accompanies an increase in the amount of substance in the preformed fibers and is the result of normal activity of the muscle, and independent of heredity. Increase of contractile substance depends least of all on inheritance and is caused by nutrition and use; as in cases of atrophy by inanition or active hypertrophy from use.

A. M. C.

**Dekhuijzen, M. C.** Becherförmige rote Blutkörperchen ("Chromokrateren"). *Anat. Anz.* **15**, pp. 206-212.

The author carried out a series of studies on the blood corpuscles of several animals, principally the brook lamprey, *Petromyzon fluviatilis*. He finds normally in the blood of this animal, which has, as is well known, circular corpuscles, red cells of a becher-like form with two poles, one rounded, convex, the opposite concave, pitted in. The eccentric nucleus lies apparently adherent to the membrane of the convex pole. The depression is sometimes so marked as to give a distinct invagination with a narrow mouth; which may be slit-like, triangular, or many angled. These cells he calls by a new name, "Chromokrateren," from analogy with Chromocyte and the Greek for crater. After a series of observations on the Arthropod *Toxichilidium* and certain mammals, upheld by observations of other workers on Pantopods and Capitellidæ, the author concludes that the becher-like cell has the form of the primitive blood cell, and hence its existence in certain primitive forms as *Petromyzon*, *Pantopods*, is most rational. A most interesting suggestion comes from observations that these becher cells of *Petromyzon* were seen to extrude their nuclei from the convex surface. Since many such shaped cells are found in the blood-forming bone marrow of mammals, the author considers it probable that

nucleated red cells may have two forms, becher-shaped and disc-like, the nuclei of the former being lost by extrusion and of the latter by absorption—in this way accounting for the conflicting statements regarding the fate of these structures.

A. M. C.

**Sargent, P. E.** The giant cells in the spinal cord, of *Ctenolabrus caeruleus* (Preliminary paper). *Anat. Anz.* **15**, pp. 212-225. 10 figs.

These giant cells were found in the cord in the median dorsal fissure in specimens that had been fixed in the following substances: 10 per cent. formol,

saturated aqueous solution of corrosive sublimate, Flemming's stronger chromic-osmic-acetic mixture, Potassic bichromate gradually raised from 2 per cent. to 5 per cent. solutions. Golgi and methylen blue failed to bring out the giant cells, though other nervous tissue was clearly differentiated. The stains most used were: Kenyon's copper sulphate-phosphomolybdic acid hæmatoxylin after formol; Heidenheim's iron hæmatoxylin after formol or sublimate; Sahl's methylen-blue acid fuchsin axis cylinder stain on bichromate material. Also, as double stains, Ehrlich's acetic acid alum hæmatoxylin and Congo red or acid fuchsin. The formol method was the most satisfactory. After killing in 10 per cent. formol and preserving in 5 per cent., tissue was washed in water and put in 5 per cent. solution of copper sulphate for twenty-four hours; it would then have a green color. Cutting in paraffin was followed by a stain made in the following way:

10 per cent. Phosphomolybdic acid	-	1 cm.
Hæmatoxylin crystals	- - - -	1 gram.
Chloral hydrate	- - - -	10 grams.
Water	- - - -	400 cm.

Sections were washed in water afterwards. By this means nerve fibers, neuroglia, and dendrites of ganglionic cells were differentiated.

Summarizing his results, the author found thirty-five to forty of these giant cells in the dorsal fissure, each enclosed in a capsule. The cells are placed in pairs. In form they are variable, with numerous dendrites which anastomose with the surrounding neuroglia cells and sometimes with dendrites of the giant cells. The cytoplasm is granular. Each cell gives off an axis cylinder which runs ventrad and laterad, dividing usually into two equal neurites, one of which enters the lateral fiber bundle; the bundle eventually passing out through the ventral root of the trigeminal nerve. Observations have shown similar cells present in the trout, sculpin, cod, eel, and yellow flounder, while they are absent in some other teleosts.

A. M. C.

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CONSIDERABLE delay in the publication of the Proceedings of the American Microscopical Society has occurred this year, due to an unfortunate combination of circumstances. Dr. Krauss, the efficient and energetic past-secretary, now president of the Society, has been ill, occasioning some delay in the transferral of the secretary's office from Buffalo, N. Y., to Lincoln, Neb., and Dr. Ward's work has also been interrupted by illness. The last pages of the volume are, however, now in the press, and will be sent to the members in April.



## NEWS AND NOTES.

An endowment has been made for furnishing laboratories for Tusculum College, Tennessee.

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The "pointer" described by "E. R." in your issue of November (p. 204) can be considerably improved by attaching it to the under surface of a ring of blackened paper or cardboard instead of cementing it directly on the diaphragm of the eye-piece of the microscope. It can then be removed when required or transferred from one eye-piece to another.

G. H. B.

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THE SATURDAY NIGHT CLUB OF MICROSCOPISTS, PHILADELPHIA.—At the February 14th meeting, Drs. T. G. Gramm and W. W. Knowlton gave an illustrated lecture on the "Minute Anatomy of the Skin and its Appendages," using the projection microscope to exhibit the microscopical preparations. The specimens shown were especially fine, many of them having been prepared for the occasion, and others brought by Dr. Knowlton from abroad.

NATHAN SMILIE, M. D., Sec.

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It is generally desirable to have students do their own staining, so far as time will permit. Most good and permanent stains, however, act too slowly to make this possible. I have been using an aqueous 1.5 per cent. solution of gentian violet with fair results, on such material as hand microtome sections of rhizomes, stems, and roots. The sections are placed in a low grade of alcohol and each student stains his own material. A section is placed on the slide and covered with a drop of the gentian violet, and after staining from two to four minutes, and dehydrating, it is mounted in Canada balsam, and studied immediately. In this way students may obtain a fairly satisfactory set of such preparations with little loss of time.

J. H. S.

Botanical Laboratory, Ohio State University.

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Prof. W. W. Alleger, Howard University, Washington, D. C., writes that he has been in the habit of using one of the meat juice extractors, which can be obtained at almost any hardware store, and which is usually sold for household use, in preparing culture media for bacteriological laboratories. The instrument is used for extracting juice not only from meat for bouillon, but also from potatoes, grapes, etc., and for the preparation of Ellsner's medium it is of great assistance. Instead of grating the potatoes and allowing them to soak over night, he cuts the potatoes lengthwise in strips an inch or so in thickness, and runs them through the meat juice extractor. The juice is caught in a beaker, while the pulp comes out as dry as a chip almost. The juice is then filtered or decanted and the requisite amount of water added. By this method the time required for soaking is saved, while the juice is extracted in a small fraction of the time required for grating and with less trouble.

ST. LOUIS MICROSCOPICAL SOCIETY, Meeting, Feb. 8.—Dr. Alt opened the scientific programme with a demonstration of blanché specimens from pigmented excrescences on iris of horse. They are common on the posterior surface, and consist to a considerable extent of connective tissue containing blood vessels and double rows of columnar epithelial cells from the pigmented areas of the iris. The specimens had been bleached in a solution of chloride of calcium containing hydrochloric acid. They had been stained with hæmatoxylin. Dr. Alt recommended the use of xylol balsam in mounting, but made use of clove oil for clearing purposes.

Dr. Bremer stated in the discussion that these growths might have a vestigial character. On December 22, 1898, the society met and listened to a paper by Dr. Ross on "The Rare Forms for Tubal Disease." Certain papillomatous growths invading contiguous tissues were not regarded by him as malignant.

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Picro-anilin-blue is used for lymphatic glandular tissue and for nervous tissues. Five cc. of the saturated aqueous solution of anilin blue are added to 100 cc. of a saturated aqueous solution of picric acid. Let the tissue remain but a few minutes in the combined solution. It is often better to use the solutions separately. Allow the tissue to remain in the blue solution until it assumes a pale sky-blue color; then immerse it in the picric solution for ten or fifteen minutes. The nuclei will have been stained a bright green and the surrounding tissue a pale pea-green. Like results are obtained in both fresh and "fixed" tissues. If the sections are to be mounted in balsam the picric stain is apt to be dissolved out by the alcohol during the process of dehydration. This can be overcome by dehydrating in alcohol in which picric acid has been dissolved. If the separate solutions have been used the tissue may be carried direct from the blue stain to a one-half per cent. alcoholic solution of picric acid, thus shortening the process.

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THE twenty-second annual meeting of the American Microscopical Society will be held at Columbus, Ohio, at about the time of the meeting of the A. A. A. S. The precise dates have not yet been fixed, but will be such as to permit those attending the meeting of the American Microscopical Society to take advantage of the reduced railroad rates which have been arranged for by the executive committee of the Society in connection with the reduced rates granted to the A. A. A. S. In selecting the city of Columbus as its meeting place, the Society accepts the invitation of the city of Columbus and the State University of Ohio, transmitted by the vice-president, Dr. A. M. Bleile. There is every indication of a very successful meeting, as the secretary has already received titles of numerous valuable papers to be presented and discussed. Reduced railroad rates are a certainty, and the interesting programmes of the A. A. A. S., and many affiliated societies whose meetings will extend over a period of two weeks, will be no small inducement to visit Columbus, which is in itself an extremely interesting city.

The eighth annual meeting of the Ohio State Academy of Science was held in Columbus, at the Ohio State University, on December 29 and 30. The meeting was well attended, and interest and enthusiasm prevailed throughout the entire session. The Academy received a gift of \$250.00 from Mr. Emerson MacMillen, which is to be used the coming year to aid in carrying on original research work and publication. The officers elected for the coming year are the following; president, Prof. G. Frederick Wright; first vice-president, Prof. C. E. Albright; second vice-president, Prof. A. D. Selby; secretary, Prof. E. L. Moseley; treasurer, Prof. Herbert Osborn; trustees, Prof. F. M. Webster, Prof. W. R. Lazenby and Prof. E. L. Moseley.

Among the large number of papers read, the following were of interest from a microscopical point of view: "The Division of the Megaspore Nucleus of Erythronium," John H. Schaffner; "Some Observations on Unio Subovatus," F. L. Landacre; "Two Interesting Filamentous Bacteria from Columbus," John H. Schaffner; "Micro-photographs of Fungus Spores," A. D. Selby and P. A. Hinman; "Development of the Microsporangium of Hemerocallis Fulva," E. L. Fullmer; "Reliability of Spore Measurements of the Fleshy Fungi," H. C. Beardslee; "Studies of Ustilago Reiliana," W. A. and K. F. Kellerman; "Further Studies in Embryology," Mrs. L. C. Riddle; "The Laboratory and the Field; their Relative Importance," H. E. Chapin; "The Illinois Biological Station," H. C. Beardslee.

J. H. S.

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PHILADELPHIA, Jan. 12, 1899.

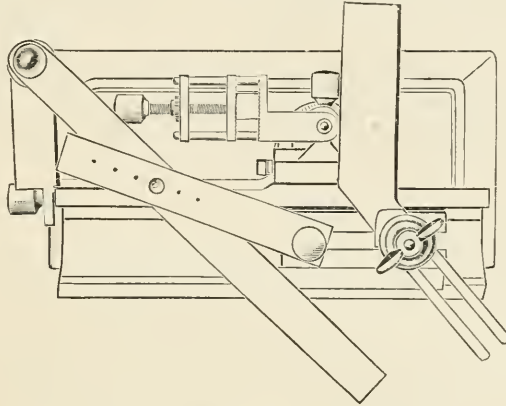
*To the Editor of the Journal of Applied Microscopy:*

Having occasionally used picrocarmine with hematoxylin, with good results as it seemed to me, I read with interest the paper on "Picrocarmine and Alumcarmine as Counterstains," that appeared in a recent number of your journal. It was also in staining developing bone, in sections of a baby's finger, that my attention was directed to the usefulness of these stains for the differentiation of other structures. In these sections the developing skin was more instructive than the developing bone. The epidermis and coiled glands were a beautiful blue, the corium was of several shades. Sections of kitten's jaw stained with hematoxylin and picrocarmine exhibited equally interesting differentiation of the young mucous membrane and the developing teeth. In the latter the dentine was rose-color, the enamel yellow, the papillæ, odontoblasts, and enamel-cells blue. In two instances I noticed a fine differentiation of epithelial elements; namely, in hair-follicles in kitten's skin, in which the cells of Huxley's layer alone were stained a deep red, other elements being blue, yellow, or light-pink; and in the testicle of a rabbit, in which the seminiferous tubules and seminal filaments were a deep blue, the tall cells of the epididymis an exquisite rose-color, their nuclei blue. I have not found it necessary, nor even advantageous, to stain with picrocarmine for more than ten or fifteen minutes. May it not be that the time required depends on the quality of the carmine, which varies much and on which the efficiency of the picrocarmine depends?

Respectfully yours,

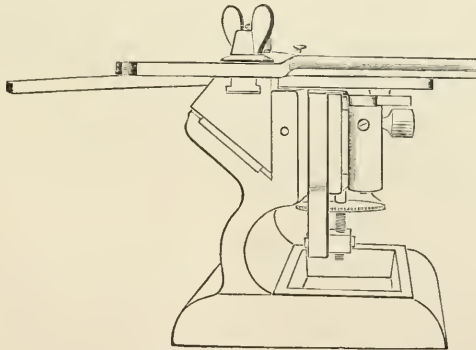
EMMA L. BILLSTEIN, M. D.

IMPROVED MOVEMENT FOR MICROTOME KNIFE.—I have made an addition to my student microtomes and also to the laboratory form, which I think is an advantage. I have met with two difficulties in using the microtome: first, the pressure of one's hand on the knife block caused the knife to change the thickness of the slice being cut; and second, in case the block of tissue presented



Top view of microtome with lever for moving knife block.

structure harder in one part than another, the knife could not be carried steadily through the whole of a section. In order to overcome the first, the knife block should be pulled in a perfectly horizontal direction, with no chance of any greater or lesser pressure downward on the way, and by using a lever with the power-arm five or six times as long as the arm attached to the knife block a steady motion of the knife could be secured.



End view of microtome showing relation of lever to knife.

The arrangement has been a very helpful one, both in my own hands and in those of my students.

THOS. D. BISCOE.

Marietta College, Ohio.

BLOOD PLATES.—An easy and sure way to procure preparations of those enigmatical bodies known as blood plates, is to place a drop of one per cent. solution of osmic acid upon the hand and with a flamed needle make a puncture through the drop of acid. The blood follows the needle and can be forced into



the drop of osmic acid without coming in contact with the air. Thus is obtained immediate and complete fixation of the blood elements. The drop of blood mixed with the acid is then placed on a slide, covered, sealed, and examined. The blood plates appear as pale yellow, minute bodies, that seem to possess more motility than the ordinary red blood corpuscle.

C. M. M.

Histological Laboratory, Cornell University.

UNIVERSITY OF PENNSYLVANIA.—Methyl-acetic stain and fixative combined has been used in cytological work on protozoa with some success. For temporary preparations, at least, the differentiation of nuclei is very rapid and clear. The formula is:

Sat. aq. sol. Methyl Green, 100 parts.

Glacial acetic, - - - 1 "

Professor Conklin has found a combination of picric acid with Delafield's hæmatoxylin to yield much better differential results than any other hæmatoxylin yet employed. For *in toto* staining it is particularly superior, as the penetration seems to be deeper and the danger of overstaining very slight—thus obviating the necessity of destaining. The picric acid stains and renders transparent the yolk in ova. This has already enabled workers who have tried it to distinguish clearly many points which are totally obscured by the clouded effects of hæmatoxylin alone. Nothing better has been found for chick embryos. The following formula gives the best results:

Delafield's hæmatoxylin, }  
Distilled water, } equal.

Picric acid, 1 drp. for each cc. of the mixture.

This may be used for a stock solution. Diluted to one-fourth and used for from ten to twenty minutes, it gives splendid differentiation in chick embryos without any necessity of decoloring.

J. R. M.

Professor J. C. Arthur of the Indiana State Experiment Station, Purdue University, recently delivered a lecture before the Biological Association of Depauw University on the biology of habit.

H. H. Z.

In reply to your correspondent's query on page 202 of the November issue, relating to the mounting of small Coleoptera and parts of insects, a paper was published on this subject by Mr. Robert Gillo in the *Journal of Microscopy and Natural Science* for July, 1885 (First Series, Vol. IV, page 151). I think it probable that Messrs. Baillere, Tindall & Cox of King William street, Strand, London, W. C. (the publishers) could supply your correspondent with a copy.

G. H. B.

Bangor, North Wales.

# Journal of Applied Microscopy.

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NUMBER 3

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## The Conditioning of Wool and Other Fibers in the Technological Laboratories of the Philadelphia Commercial Museum.

Competition and the tremendous industrial development of the present century have injected into commerce a new element which may be designated as the element of exactitude.

Time was when the span of the hand, the length of the arm, or the length and weight of the barley-corn were considered sufficiently uniform to serve as standards for all ordinary purposes of weight and measure. All this has changed, and absolute accuracy has been substituted for absolute inaccuracy. As alchemy and astrology, shadowy, mysterious, superstitious, and nearly meaningless, have given place to chemistry and astronomy, definite, certain, and based on known laws, so nearly every kind of industrial and commercial activity has been gradually developing along lines of greater and greater accuracy of workmanship, methods, and knowledge. The supplanting of the "rule of thumb" by methods and instruments of precision has been one of the remarkable features of the present era, and so important a bearing does accuracy have upon the success of an enterprise that a knowledge of the methods and means of obtaining exact information is now considered one of the prerequisites to the investing of capital.

The buying and selling of wool, however, is still done almost entirely by the rule of thumb, but this bids fair to give place to the more accurate method known as "conditioning," i. e., the application of instruments and processes of precision to determine the physical condition of the wools, as for instance, the average diameter of the fiber, the average length of the staple, the strength, elasticity, and probable age, the amount of dirt, grease, potash, and moisture, the breed of the wool, adulterants, etc.

An idea of the importance of the subject may be gained from the following report of a test, quoted from the records of the Philadelphia Museum's wool-conditioning laboratory:

Sample No. 1065, submitted by ——— contained:

Moisture,	-	-	-	-	10.06 per cent.
Grease and Dirt,	-	-	-	-	43.03 "
Available Wool Fiber,	-	-	-	-	46.91 "

This means that 100 pounds of "wool" contained only 46.91 pounds of wool.

Sample No. 596, a very extreme case, yielded only 26 per cent. of wool fiber. The index of strength and elasticity may vary from normal to practically nothing.

It is intended in this series of articles to describe the Museum's work in the practical examination and investigation of fibers, this first paper being restricted to some hints regarding the application of the microscope to commercial fiber investigation.

The study of the minute structural features of any material must, of necessity, be attended with considerable difficulty, and, in this respect, the examination of wool fibers offers no exception to the rule.

There are but few existing rules, laws, methods, and processes to serve as guides in the work. In a few isolated cases some facts have been recorded concerning tests made in Germany, England, France, and Russia, but these facts have not been definitely correlated, and only stray references have been made to the methods and instruments of research. The Technological laboratories of the Philadelphia Commercial Museum were established for the purpose of furnishing impartial and reliable tests, not only of wool, but of all other kinds of fibers, whether of animal or vegetable origin.

Work of this kind, especially when made the basis of a guarantee of absolute reliability, has been accompanied by serious difficulties, the most prominent of which has been the absence of precedents in this country, and the consequent lack of reliable testing instruments and approved methods of conducting an investigation intended to supply manufacturers and others with accurate data relating to the essential and actual value of wool and other fibers and textile materials.

It was, in fact, necessary to devise special machinery and apparatus for the testing, treating, and measuring of the fibers; and instruments designed for the one class of fibers will not do for all, since each kind requires a different method of investigation.

The most valuable instrument in studying the special forms and structures of fibers is the microscope, with its various attachments for obtaining exact views and accurate measurements. The fibers of wool, when seen through a microscope, are seemingly so uniform and transparent that, in order to become familiar with the characteristics and variations of the diverse types and breeds, it is advisable to use a microscope of high power and clear definition. Optical conditions being favorable, some wools, hairs, and furs will show numerous irregular lines crossing the fiber. The edges of the fiber may appear perfectly regular, or they may be more or less serrated, the number and size of the teeth varying according to the breeds from which the samples are selected. This slight variation, and the nearly amorphous and transparent appearance of the wool render it necessary to make repeated tests upon each class of wool, until the observer becomes fitted by experience to identify the various breeds, qualities, and stages of manufacture.

The color, resulting from pigment contained in the center of the fiber of certain breeds, furnishes a fairly reliable test, when the observer has learned by experience and practice to distinguish the slight, yet distinct, differences in the

various pigments. The various manufacturing processes, such as scouring, carbonizing, bleaching, dyeing, etc., effect great changes in the wool fiber, and the observer should make himself familiar with all of them.

To cite an extreme case, a weak solution of caustic soda (Na. H. O.) will transform the wool fiber into an opaque, somewhat swollen, gelatinous body, while a strong solution will dissolve it entirely. The various reagents should be separately applied and the effects noted, for some will produce partial, others complete disintegration, while frequently other changes fully as striking occur.

Some wool fibers are cylindrical and are covered with somewhat irregular scales, or with bundles of elongated fibers of epithelia, while the wool fibers sometimes show cellular cavities or canals filled with granules of pigment. These are some of the principal characteristics of the wool fiber which, together with careful study, will enable the observer to classify the wools as to their breeds and grades, commercial values and conditions.

In illustrating the features mentioned, the American Merinos will serve as a type. The wool of the Merino sheep is comparatively easily distinguishable from that of the Lincoln, Cotswold, and Oxford breeds, but not so easily from the Southdown, and much Southdown wool has been, and is, sold for Merino, so closely do the wools of these two breeds resemble each other, and so easily are they confused with each other.

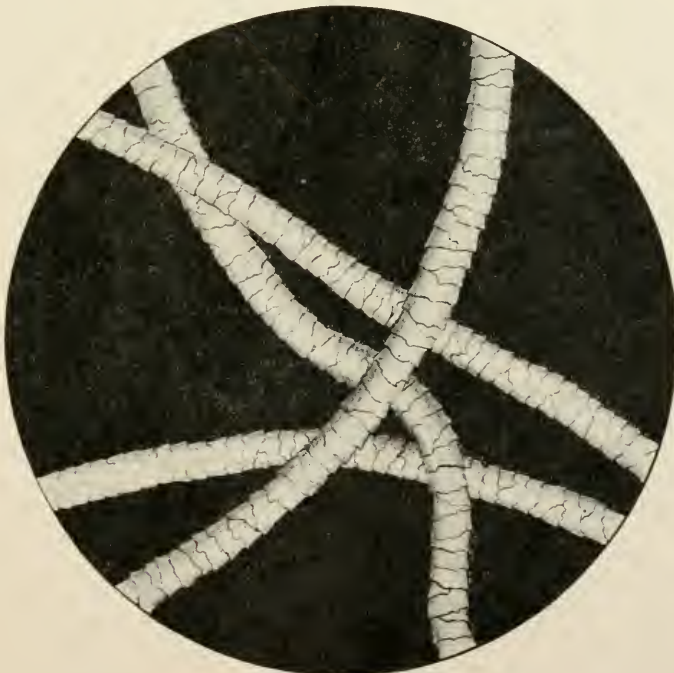


Fig. 1.

For example: Fig. 1 represents a fiber of American Merino wool drawn from solar projections x 180.



Noticing the scale-like structure of the fiber, as shown by the sketch, let us enquire into the peculiarities of these scales. For this purpose place a number of Merino fibers upon a glass slide, and cover them with another slide of equal thickness. Apply one or two drops of ( $\text{H}_2\text{SO}_4$ ) sulphuric acid (c. p. S., G. 128°) to the edge of the glass cover, so that the liquid will be drawn between the glasses by capillary attraction. Place the slide under the microscope, and a one-inch objective will serve to show the changes the fibers will undergo from the action of the reagent. As soon as the sulphuric acid is drawn over, and impregnates the fibers, they are observed to swell or expand slightly, and transverse markings become apparent. When no more changes occur, and the reaction thus seems complete, the slide should be removed and heated with an ordinary alcohol lamp until it becomes perceptibly warm to the hand. It should then be replaced upon the stage of the microscope, and the changes noted. It will be seen that the simple heating of the slide has caused the transverse markings to become decidedly more prominent and the serrations at the edges of the fibers much more distinct. Thin scales (epidermal epithelia) begin to separate slowly by curling up along their edges, and these in reality caused the transverse markings. They now separate from the main structure, and when entirely free, show a tendency to curl themselves up and roll into little spheres, totally unrecognizable, and permitting of no further profitable study. At present there is no means of determining the form of these scales in this separated, free condition, but as studied while in the act of separating from the fiber, they are unequal, distorted, very transparent, and extremely thin, having a thickness of only 0.0014 millimeters. There can be little doubt that nature designed these little scales to give wool fiber its peculiar felting property, a characteristic upon which the value of the staple for manufacturing purposes so largely depends.

ERNST FAHRIG, Ph. D.,

Chief of Laboratories.

WILLIAM P. WILSON, Sc. D.,

Director.

## The Preparation of Ground Sections of Teeth and Bone.

Few preparations for the microscope demand the painstaking attention to manipulative details that is required for the successful preparation of fine sections of teeth and bone. Many short notices have appeared in books and journals on rapid methods of making these sections by hand; rubbing them down on glass, stone, and in other ways. I have tried many of these and do not find that I can get good results. To those who simply want one or only a few sections, I would say, you can get them from a dealer far cheaper than you can make them. To the student, or those who desire to make many sections, I offer the method I use, to which I have drifted after large experience in making these preparations.

*Apparatus.*—A grinding machine, suitable laps of copper, lead, and stone, grinding powders, a chemist's wash bottle, a small metal table, hardened balsam. My grinding machine is home-made. It is a large Swiss jeweler's lathe, fitted

with a screw chuck to carry laps, so arranged that the laps screw to a seat with great perfection. Above and parallel to lathe spindle is a shaft in adjustable bearings; from this depends an arm or carrier, the face of which is arranged to receive and clamp with great accuracy cast-iron grinding blocks, about  $3 \times 2 \times \frac{1}{2}$  inches in size. I use lead, copper, and "Scotch Water of Ayr" laps, which are turned true on this lathe. These laps after turning are ground one against another to *perfect* truth of surface. The grinding block, which when in the carrier can swing and be withdrawn from the lap, but is held truly parallel to the face of the lap, is now ground perfectly true to the lap. I use carborundum in four grades, from grains to that which requires fifteen minutes to settle. I grind with water from a chemist's wash bottle. Use the grains on the lead lap, the powders on the copper, finish with stone lap and water. The process can be divided into four heads: (1) preparation of material; (2) imbedding; (3) grinding; (4) mounting.

1. *Preparation of Material.*—(a) Teeth work best that are started "green," i. e., that have never been allowed to dry out, for drying develops many small cracks, especially in the enamel. If the teeth need cleansing, wash them in dilute lye. Preserve in turpentine. Make rough sections with a ribbon or hack-saw. Make these sections thick, not less one-sixth the length of the section, if possible. Where the saw cannot be used, grind away half of the tooth and make the section of the remaining one-half. True the surface roughly which is intended for the section. Wash carefully, picking out any grains of carborundum, and removing any soft tissues that it is not specially desirable to leave, as these are very apt to cause trouble. (b) Bones: Secure, if possible, fresh bone. Cut into short pieces, and macerate in water till all soft tissues are removed. Bone from the dissecting hall, where subjects have been injected, is not well suited for this purpose, though, when well cleaned and selected, gives very good results. Saw the bone into rough sections as desired, with the hack-saw, being careful that the thickness is never less than one-sixth the greatest dimension.

2. *Imbedding.* The material, after being washed and cleansed of all grit and dust, is dehydrated in alcohol. For imbedding as well as for cementing the sections to grinding blocks, prepare some hard balsam. Take a commercial grade of balsam and dry it over a gentle heat till, when cold, it will receive the impression of the finger nail under steady pressure, but flies to pieces under shock. The material being dehydrated, pass it into chloroform; after a day add hard balsam from time to time for a week or ten days till the solution is syrupy, then pour off the solution and dry the material for a week or more, so that when cleaned of surplus balsam it shows no stickiness.

3. *Grinding.*—(a) Teeth: Clean away from the material all surplus balsam. Coat the surface of a grinding block with hard balsam. Arrange on this, section surface down, as many sections as can be placed on the surface, warm on the metal table, not to boiling, press each section carefully down into its place, cool, put into carrier, grind with coarse powder till a supporting surface is developed, supporting as nearly as possible the whole of each section surface. True this supporting surface accurately. Take a second grinding block, heat on the metal

table, coat with balsam, remove to a wooden support, press into this softened balsam quickly and accurately, using only as much pressure as can be given with the hand, the supporting surface of sections. Before the sections can become heated through, plunge the whole in cold water; when thoroughly cold, wipe off and place on hot table with first block down. As soon as the water between the blocks begins to boil, slip block No. 1 quickly off and plunge block No. 2 with sections into cold water. Place the block into the carrier, grind with medium powder till the sections all show a surface suitable for finished sections. Polish the surface with finest powder, then with the stone lap, and finally with dry whiting powder and the hand. After polishing carefully, wash with water, being careful to remove all powder or grit of any kind, dry the surface carefully, and wipe the surface with the finger dipped into gasoline, using only the least possible quantity of gasoline. Clean block No. 1, heat it, and coat with balsam. Transfer the sections to block No. 1, as outlined above, being very careful to exert a uniform pressure in bringing the sections down on block No. 1. Grind first with the grains of carborundum, then, as the sections get thin, use finer and finer powder. If too coarse a grade of powder be used as the sections get thin, the enamel will be broken and the sections will leave the block. When all color in the enamel begins to disappear, use the stone lap, then grind till all color is gone from the enamel, the dentine looks transparent, not white, and the block seems to have only a coat of varnish on the surface. Polish with the palm of the hand and whiting, wash in water, dry, wash in gasoline, dry, immerse in benzole till the sections come away. (*b*) Bone is prepared in the same way, save that each section is roughly trued on one side before cementing to the block; they are then cemented with trued surface down, and the section surface is prepared directly, making only one transfer necessary in preparing the sections.

4. *Mounting*.—Tooth and bone sections may be mounted either dry or in balsam. If the cells do not suffer with a deposit of "dew," the dry mounts are the more permanent. The balsam mounts, if carefully made, will last in good condition for many years, and I think are much finer. I prepare cells for dry mounts with gelatine, and seal them with a fine ring of a hot thick solution of gelatine, finishing in black or colors, as desired. It is essential that the sections shall perfectly dry, the cell dry, and both quite warm when the cell is closed. When made in this way I have lost few slides from "dewing." For balsam mounts I use paper-filtered balsam. Dry this on a slide, in large excess, till when cold it is nearly hard, but not flinty. While hot, coat with this one side of a suitable cover-glass, allow both to cool. Place the section between the two and press the cover down, using as little heat as possible. With a little practice and dexterity all the air bubbles that show up about the section will be washed away. Too much heat fills the section and ruins the mount. Cut away the excess of balsam, and wash, first with gasoline, then with soap and water.

As I have said, success depends upon *attention to details*. One grain of grit out of place at a critical moment will spoil all the sections. I have ground over one hundred sections of small teeth on one block at one time. I usually grind twenty-five longitudinal sections of human teeth at once and I have made

sections of teeth three inches in length and less than  $\frac{1}{1200}$  inch thick by this method. The same method can be used for any ground sections, applying of course suitable modifications, e. g., spines of Echinie, soft and hard tissues imbedded according to Von Koch or Weil, minerals, etc.

S. P. COWARDIN.

March 1st, 1899.

## Methods of Making Microscopic Preparations of Copepoda.

Although the copepods are present everywhere and are very easily collected, few books, so far as I know, give directions for preparing and mounting them for the microscope. It is true that the dissection of these minute animals requires much patience and some skill, but many more difficult operations are undertaken even by amateurs. It seems a little strange, therefore, that there are so few students who have even an elementary knowledge of them.

Copepoda and entomostraca in general are most easily collected by means of the dredge devised by Professor Birge, the essential part of which is the mouth covered with a cone of coarse wire gauze. This gauze-covered mouth keeps out the sticks, weeds, etc., which otherwise would immediately fill the net, so that the net can be repeatedly drawn through a weedy pond, and a large collection can be made which is comparatively free from débris.

If the copepods are to be used for histological purposes, they should be killed by some one of the osmic acid preparations. If, however, they are to be used for systematic purposes, or for the study of the muscles, I have found no killing and preserving agent so good as alcohol. Formalin leaves the material brittle and unfitted for dissection, and is, besides, excessively disagreeable to the one who has to work over it. The collection can be placed immediately in alcohol, the water which will go with it diluting it sufficiently, and nothing further need be done with it until one is ready to study the material.

In order to stain the material, most of the alcohol should be withdrawn by means of a pipette, and a little picro-carmin put into the bottle. The best results are obtained by a somewhat prolonged exposure to a dilute stain—from one to three days. Anilin stains may be used, but picro-carmin is more satisfactory. For a systematic study of the copepoda, it is necessary to dissect off the various appendages and mount them in order. This requires a great deal of patience and a steady hand. I suppose that it is this work which deters so many from attempting to do anything with these animals. The dissection must be done on the slide, and, inasmuch as alcohol evaporates very rapidly, it is best to replace the alcohol with glycerine. This change from alcohol to glycerine must be made very gradually, or the specimen will be distorted and perhaps ruined. The finest needles must be used for the dissection, and better work can be done if the needles are carefully ground down on two sides so as to make minute scalpels.

The work of dissection on the larger forms can be readily carried on under an ordinary dissecting lens, but for the smaller forms it is almost necessary to



have a lens with which a power as high as one hundred diameters can be obtained.

Balsam is a very unsatisfactory mounting medium for copepods, and I use almost exclusively for this purpose Farrant's medium. The parts as dissected in glycerine can be removed directly to this medium. If one is careful to spread out the drop of Farrant's on the slide in a thin layer, the parts can be placed in order, and the cover-glass put upon them with very little disturbance of their relative positions, and it is not necessary to use anything to fix their positions on the slide.

The covers can be ringed with any good cement. I use mostly Brunswick black. The shrinking of the Farrant's will sooner or later cause the cement to crack, so that it is necessary to ring them again after some months, if the preparations are to be kept permanently. The preparations do not suffer, however, if this second ringing is neglected, as the Farrant's hardens, and the preparations will keep perfectly except as they are subject to hard usage.

C. DWIGHT MARSH.

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## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

### I.

During recent years the microscope has been finding its way into a constantly increasing number of schools, colleges, and homes, and, as might be expected, there has been an increasing demand for better methods of preparing material for microscopic study. The earlier investigators examined objects in the dry condition and without cover glasses. If a thin piece of pine shaving be examined dry, it shows some structure, but it shows more if wet with a drop of water and covered with a cover-glass, and it shows infinitely more if properly stained and mounted.

It is the purpose of this series of papers to present the current methods of preparing botanical material for microscopic examination, and in presenting the subject we shall try to meet, as nearly as possible, the needs of the amateur, the teacher, and the student engaged in research work. Methods will be described for mounting all kinds of material, from the lowest algæ to the highest flowering plants, but the order in which the methods are taken up will be governed entirely by the comparative difficulties of the technique, it being assumed that the reader is a beginner. While very few of the methods are original with the writer, it is nevertheless true that the descriptions are based upon a somewhat extensive experience in laboratory work with beginners and with advanced students.

After a brief mention of the apparatus and reagents needed for the work, attention will be given to the various methods of killing, fixing, hardening, dehydrating, imbedding, sectioning, staining, mounting, drawing, etc. The paraffine method and staining will receive particular attention.

## APPARATUS.

The following list of apparatus includes a fair equipment for histological work: a microscope magnifying at least 400 diameters; a hand microtome; a sliding microtome; a razor; a paraffine bath and lamp; a turn-table; a scalpel; a pair of needles; pair of scissors; pair of forceps; stender dishes; minots or watch glasses; a wash bottle; a graduate (50 or 100 cc.); pipettes; slides, 1 x 3 inches; round covers, 18 mm. or  $\frac{3}{4}$  inch in diameter; and square covers,  $\frac{7}{8}$  inch.

The hand microtome will be found extremely useful, especially by the busy teacher who has large classes. Any sliding microtome, if kept in good order, will be sufficient for the work to be described in these papers, but those of medium size are to be preferred.

The stout razors our grandfathers used to shave with are excellent for free-hand sectioning, for hand microtome work, and even for cutting paraffine sections on the sliding microtome. The blade should be flat on one side (Fig. 1 A). Modern razors (Fig. 1 B) with delicate blades, though good to shave with, are worthless for cutting sections of plants. The razor is a necessity: if a microtome knife is wanted in addition, it should have a bevel about like Fig. 1 A. A short blade, three or four inches long, is to be preferred to the longer ones, which are much more troublesome to sharpen.

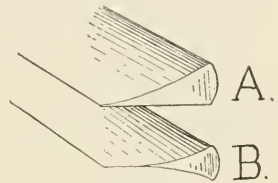


Fig. 1.

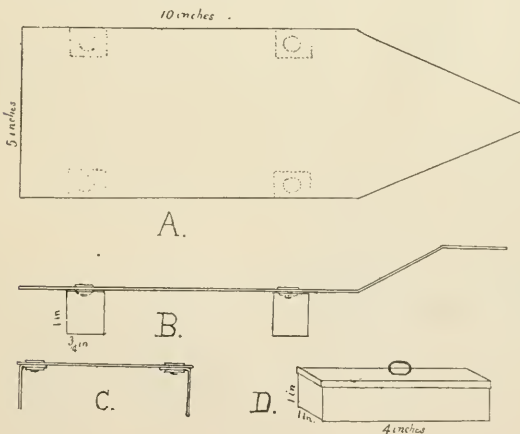


Fig. 2.

A, top view; B, side view; C, end view; D, box to contain the paraffine.

There are numerous forms of the paraffine bath. Those with a water-jacket, thermometer, and a thermostat, to maintain an even temperature, are the most convenient where gas is available. As a rule it is easier to keep the temperature constant in the larger baths. A bath which, if carefully watched, gives the very best results, can be made by any tinner, and is very inexpensive. The accompanying figures show the form and dimensions.

It is made of copper one thirty-second of an inch thick, but thicker copper is as good or better. There should be two boxes to contain the paraffine; the covers to the boxes should fit loosely. Any kind of a lamp may be used.

Stender dishes are now very generally used for staining on the slide. The form shown in Fig. 3 A is made just large enough to hold two slides, placed

back to back, and hence requires only a minimum of the reagent. The cap in this form does not fit closely enough to keep absolute alcohol and xylol, but

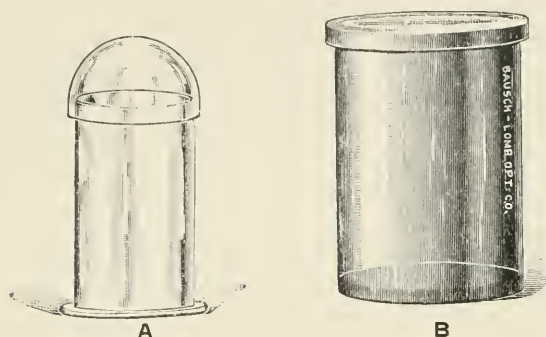


Fig. 3.

does very well for the other alcohols and stains. The form shown in Fig. 3 B is the best for absolute alcohol and xylol, but even with this it is better to put a little vaseline or glycerine on the cover to prevent any evaporation. Wide-mouthed bottles, though not so convenient, give just as good results.

The other pieces of apparatus mentioned need no comment. By consulting a catalogue, which will be furnished by any dealer, the beginner can determine what he needs to buy, and what he can find substitutes for, if it is necessary to be very economical.

#### REAGENTS.

It would require entirely too much space to even enumerate the reagents which are occasionally used in a fully equipped university laboratory. The following list includes only those which are used constantly. The quantities given indicate about what the average student uses in a three-months course in methods. Nearly all the stains, however, would last for a year if properly used.

**KILLING AND FIXING AGENTS.**—Commercial alcohol (about 95 per cent.), 2 liters; absolute alcohol, 200 cc.; ether, 50 cc.; chromic acid, 10 g.; corrosive sublimate, 10 g.; glacial acetic acid, 25 cc.; hydrochloric acid, 50 cc.; picric acid, 5 g.; chloroform, 50 cc.; [osmic acid, 1 per cent. solution in water, 100 cc. This is extremely expensive, and not necessary except for the most delicate work]. Formulæ for making killing and fixing agents from these materials will be given later.

**STAINS.**—Only a few of the most important stains are given in this list. In general, one should have enough of a stain to stand about two inches high in the Stender dish, or bottle in which the staining is to be done. The theory and practice of staining will be discussed in a future paper.

*Delafield's Hæmatoxylin.*—To 100 cc. of a saturated solution of ammonia alum add, drop by drop, a solution of 1 g. Hæmatoxyn dissolved in 6 cc. of absolute alcohol. Expose to air and light for one week, then filter. Add 25 cc. of glycerine and 25 cc. of methyl alcohol. Allow to stand until the color is rather dark. Filter, and keep in a tightly stoppered bottle. The solution should stand for two months before it is ready for use, but, if needed immediately, the "ripening," which is brought about by the oxidation of hæmatoxylin into hæmatin, may be secured in a few minutes by a judicious addition of peroxide of hydrogen.

*Mayer's Hæmalum.*—Dissolve with gentle heat 1 g. of hæmatoxylin in 50 cc. of 95 per cent. alcohol; add a solution of 50 g. of alum in a liter of dis-

tilled water. Allow the mixture to cool and settle; filter; add a crystal of thymol to preserve from mould. The stain is ready for use as soon as made, and it keeps well.

*Haidenhain's Iron Alum Hæmatoxylin.*—Two solutions are used, and they are never to be mixed.

(a). A  $1\frac{1}{2}$  to 4 per cent. aqueous solution of ammonia sulphate of iron.

(b). A  $\frac{1}{2}$  per cent. aqueous solution of hæmatoxylin.

*Cyanin, Erythrosin, Safranin, Gentian Violet.*—Numerous formulæ are given for these and other anilin stains, but the following general formula gives excellent results:

Make a 3 per cent. solution of anilin oil in distilled water; shake thoroughly and frequently for a day; add enough alcohol to make the whole mixture about 20 per cent. alcohol. Add 1 g. of cyanin or erythrosin, etc., as the case may be, to 100 cc. of the solution. Safranin is often used in strong alcoholic solution, and even with the above formula it is better to dissolve the safranin in strong alcohol before adding it to the mixture.

*Acid Fuchsin.*—Use a 1 or 2 per cent. solution in water, or 70 per cent. alcohol.

*Iodine Green.*—Use a 1 to 4 per cent. solution in water or alcohol. A 3 per cent. solution in 70 per cent. alcohol is very good for the vascular system of plants.

*Mixtures of Fuchsin and Iodine Green.*—The following formula is often used for karyokinetic figures:

(a). A  $\frac{1}{2}$  per cent. solution of fuchsin in water.

(b). A  $\frac{1}{2}$  per cent. solution of iodine green in water.

Just before using mix *a* and *b* in various proportions until you find what is needed for the particular case.

*Orange G.*—Use a saturated aqueous solution.

*Eosin*—A 1 to 5 per cent. solution in water or alcohol. A 2 per cent. aqueous solution is good for material to be mounted in glycerine, but a 2 per cent. solution in 70 per cent. alcohol is better for balsam mounts. The stronger solution may be diluted as needed for special cases.

FORMULÆ FOR ALCOHOLS.—The grades of alcohol in most common use are 35 per cent., 50 per cent., 70 per cent., 85 per cent., 95 per cent., and 100 per cent. The 100 per cent. is expensive, and great care should be taken to keep the bottle well corked or the stender dish closely covered. The following formulæ will enable any one to make the other grades of alcohol from 95 per cent. alcohol and water:

95	35	95	50	95	70	95	85
	—		—		—		—
	60		45		25		10

The above are the formulæ for 35 per cent., 50 per cent., 70 per cent., and 85 per cent. alcohol. Any other grade can be gotten in the same way. In the first formula, subtract 35 from 95; the result, 60, is the number of cubic



centimeters of water which must be added to 35 cc. of 95 per cent. alcohol in order to obtain 35 per cent. alcohol. The mixture contains 95 cc. of 35 per cent. alcohol. If more or less than 95 cc. of the mixture is needed, take proportional parts of 35 and 60. This simple method is a time saver, but if the bottles or Stender dishes are to be filled frequently, it will be a still further saving of time to use a long label (Fig. 4), and after pouring in the 95 per cent. alcohol draw a line showing how high it reaches, and then, after pouring in the water, draw another line. The next time it is necessary to fill the bottles, merely pour in 95 per cent. alcohol until it reaches the first line, and then pour in water until it reaches the second line. It is better, but not absolutely necessary, to use distilled water.



Fig. 4.

**CLEARING AGENTS.**—Xylol is the most generally useful clearing agent yet known. Clove oil, cedar oil, bergamot oil, carbolic acid, and turpentine are all necessary for special purposes. About 125 cc. of xylol and 25 cc. of each of the others makes a fair outfit to begin with.

**Miscellaneous.**—Paraffine (hard and soft), celloidin, Canada balsam, glycerine, glycerine jelly, gold size, or any good sealing medium.

**Arrangement of the Outfit for Staining and Mounting.**—It is best to keep the various reagents in definite positions in order that no time may be lost in hunting for anything. The following diagram (Fig. 5) of a part of the top of a table shows a convenient arrangement.



Fig. 5.

The alcohols are in front and the stains are placed behind. The eosin, fuchsin, iodine green, cedar oil and clove oil may be kept in bottles; the rest should be in Stender dishes.

(To be Continued.)

THE Zoölogical Club of the University of Nebraska is an organization of graduate students, which meets weekly for the discussion of specific problems and topics which are assigned to various members. During the past semester the club has been studying the development and progress of plankton investigations.

## A Method for Making the Three Principal Artificial Media, Based on the Bacteriological Committee's Recommendations.

It is scarcely necessary, at this date, to recapitulate the reasons for the use of simple, and particularly of exact, methods in the preparation of culture media.

The first important impulse in this direction was given by the Convention of Bacteriologists, summoned by the Committee on Water Supplies of the American Public Health Association in 1895. This convention, the proceedings of which were published in the October number of the Journal of that Association, appointed a Bacteriological Committee, including a number of the best known bacteriologists in America, empowered to draw up a series of methods selected from the best extant at the time which should be recommended as standards, particularly for species differentiation.

The writer, having been associated with a member of the committee who dealt particularly with the question of the preparation of nutrient broth, gelatin, and agar-agar, was able to suggest certain minor changes in the tentative methods published in 1895, which were incorporated in the recommendations of the Bacteriological Committee, published finally in the Journal of the American Public Health Association, January, 1898.

Further work on the subject has led the writer to suggest here a slight change in the preparation of nutrient broth, designed to prevent the precipitation of albumens during sterilization which may occur if the methods of the Bacteriological Committee are rigidly followed. The tabulation of the necessary steps, given below, will demonstrate this point clearly. Step No. 14, in this table, is recommended by the Bacteriological Committee to follow Step No. 10. The writer has placed it in its present position for the reason already given.

In June, 1898, Ravenel published in *THE JOURNAL OF APPLIED MICROSCOPY* an excellent method for the preparation of nutrient agar, certainly the best which has yet appeared. The writer, working independently along similar lines since 1895, arrived at a quite similar method, which has an advantage over that of Ravenel, however, in that it does not require the use of the autoclave. In the table, this method has been so developed as to place the preparation of the three principal media on an analogous basis, at the same time adhering closely to the methods and spirit of the Bacteriological Committee's recommendations. (See table.)

A question naturally arises as to whether or no the extraction of meat with an equal quantity of water, as here recommended for nutrient agar, will yield the same results as the extraction of the same quantity of meat with twice its weight of water, the method usually employed. The writer has found that the former process yields a slightly smaller amount of total solids. The estimation was made for the writer by Dr. Charles Harrington, of the Laboratory of

Hygiene of Harvard Medical School. The difference in percentage composition lies in the second place of decimals, and is therefore of little moment.

Every step given in the table (except Step No. 4) is necessary to exact uniformity in results. For instance, if Step No. 11 be omitted, the reaction may be adjusted to +1.5 accurately enough, so that the acid contents of various successive lots of media will be uniform, but the degree of concentration of the *other* constituents, depending as it does on the loss through evaporation during Step No. 10, is almost certain to vary from lot to lot, for the extent of the surface of media exposed to the air affects the amount lost. Thus 500 cc. of media will lose more if cooked in a wide, shallow vessel, than it will lose in a narrow, deep vessel. Moreover, a greater *percentage* of loss by evaporation occurs in preparing 500 cc. of media in one vessel than in preparing 2000 cc. of the same media in the same vessel.

In Step No. 16, it is to be noted that the cotton itself filters out only the bulky coagulum formed in the cooking processes, No. 10 and No. 14. This bulky coagulum, however, once deposited on the cotton, forms thereafter an extremely good filtering material, and ensures a perfectly clear filtrate, if the filtrate be passed through it two or three times.

Step No. 15 is necessary, because a reaction adjusted to +1.5 per cent. will rise if the media be subsequently concentrated. It is to be noted, however, that in adjusting the reaction to +1.5 per cent. by Fuller's method (addition of normal acid solution) a certain quantity of water is added at the same time—the water of the normal solution—so that the five minutes boiling (Step No. 14) usually brings the final weight of the finished medium to about the original weight of the infusion.

The writer holds strongly the view, based upon comparative bacterial counts, that the practice of sterilizing media in bulk, with the object of subsequent successive removal and use of small portions, is to be condemned. The media in bulk, left after the withdrawal of these portions, generally requires further sterilization after each withdrawal. The portion withdrawn also generally requires sterilization. Hence the later portions withdrawn receive an amount of sterilization not usually given to media tubed and sterilized directly after preparation. Additional sterilization means really additional cooking. If one lot of media be cooked twice as long as another, there can be little doubt that the composition of the two lots will differ somewhat. It is true that the exact series of changes which take place amongst the many delicate chemical compounds present in meat extracts during the preparation of media are not known. On the other hand, a rigid uniformity of technique will ensure that, as far as possible, the *same* series of changes (whatever the changes may be) will be obtained in every lot of media prepared. This is as far as any one can go at the present time. No one should be content with anything short of this.

In sterilization, therefore, an exact degree and length of time of exposure to heat should be adopted. Fifteen minutes in flowing steam on three successive days will sterilize any liquid or liquified media in tubes. More than this is unnecessary, except where media in bulk are to be sterilized. Prolonged heating is then necessary to ensure the proper temperature throughout the whole mass.

Flowing steam is perhaps preferable to the autoclave, inasmuch as chemical changes are less likely to occur at 100°C. than at higher temperatures. It is best to make certain that the sterilizer is full of steam before putting the tubes into it, otherwise the prolonged gradual rise from the cold water temperature (which is likely to vary in different cases) to the boiling point introduces another, although minor, discrepancy. Similarly, the media should not be left in the sterilizer to cool down for indefinite periods. It is best to turn out the gas and remove the media as soon as the fifteen minutes sterilization is accomplished. Nutrient gelatin tubes should be plunged at once into ice water to ensure the maintenance of a high melting point. A melting point of 25°C. can thus be uniformly obtained without difficulty for 10 per cent. gelatin.

The use of prepared extracts of meat is steadily giving way to the use of meat infusions; these methods may, however, be adapted to their use also. Perhaps the simplest adaptation is the following:

Mix thoroughly the white of one egg with 1000 cc. water (for nutrient broth and gelatin) or of one egg to 500 cc. water (for nutrient agar). Proceed then, beginning with Step No. 5, exactly as given in the table, substituting "white of egg solution" for "infusion" wherever the latter term occurs, but at Step No. 7 add also 0.5 per cent. of Liebig's extract for nutrient broth or gelatin, or 1.0 per cent. for nutrient agar. This proceeding will result in a liter of each medium.

The titrations necessary are made by Fuller's method. Phenolphthalein is used as the indicator: "+" indicates acid; "-" indicates alkaline."

The methods of titration, and the reasons for the adoption of these methods are given in the following:

Journal American Public Health Association, October, 1895.  
Massachusetts State Board of Health Report, 1895 (Fuller and  
Copeland).  
Journal American Public Health Association, January, 1898.

TABLE SHOWING ANALOGY BETWEEN BROTH, NUTRIENT GELATIN, AND NUTRIENT AGAR,  
MADE BY METHODS HEREIN RECOMMENDED.

NUTRIENT BROTH.	NUTRIENT GELATIN.	NUTRIENT AGAR.
1. Infuse lean meat twenty hours with twice its weight of distilled water in refrigerator: Say 1000 grams meat, 2000 grams water.	Ditto.	Boil thirty grams thread agar in one liter of water for half an hour. Make up loss by evaporation to a weight of 1000 grams. Cool and solidify.
2. Make up weight of meat infusion (and meat) to original weight by adding water, i. e., to 3000 grams.	Ditto.	Infuse lean meat twenty hours with <i>its own</i> weight of distilled water in refrigerator. Say 1000 grams meat, 1000 grams water.  Ditto. i. e., to 2000 grams.



NUTRIENT BROTH.	NUTRIENT GELATIN.	NUTRIENT AGAR.
3. Filter infusion through cloth to remove meat.	Ditto.	Ditto.
4. Titrate and record reaction of filtrate. Say reaction $\pm 2.2$ per cent.	Ditto.	Ditto. Say reaction $\pm 4.2$ per cent.
5. Weigh infusion. Say 1800 grams.	Ditto.	Ditto. Say 900 grams.
6. Set infusion on water bath, keeping temperature below $60^{\circ}\text{C}$ .	Ditto.	Ditto.
7. Add peptone, 1 per cent. 18 gr. Add salt, 0.5 per cent. 9 gr.	Ditto, and sheet gelatin 10 per cent. 180 gr.	Add peptone, 2 per cent. 18 gr. Add salt, 1 per cent. 9 gr.
8. After ingredients are dissolved, titrate; reaction probably $\pm 2.3$ to $\pm 2.5$ .	Ditto. reaction probably $\pm 4.0$ to $\pm 5.0$ .	Ditto. reaction probably $\pm 4.5$ to $\pm 4.7$ .
9. Neutralize (Fuller's method).	Ditto.	Ditto.

To the 900 grams of meat infusion (containing now peptone and salt also) add 900 grams of the 3 per cent. agar jelly described at head of this column. Since agar is neutral, the reaction is unchanged.

10. Heat over boiling water (or steam) bath thirty minutes.
11. Restore weight lost by evaporation to original weight of filtered meat infusion, i. e., that on which the per centage of peptone, salt, etc., were calculated—1800 grams in *each case*.
12. Titrate—reaction probably  $\pm 0.3$  to  $\pm 0.5$ .
13. Adjust reaction to final point desired—generally to  $\pm 1.5$  per cent.
14. Boil five minutes over free flame, with stirring.
15. Add water, if necessary, to make up loss from evaporation to 1800 grams.
16. Filter through absorbent cotton, passing the filtrate through the filter repeatedly until clear.
17. Titrate to determine whether or not the desired reaction has been maintained.
18. Tube and sterilize.

HIBBERT WINSLOW HILL.

Director, Laboratory of the Boston Board of Health.

A BLACK FINISH FOR TABLE TOPS.—Professor J. R. Slonaker gives a formula taken from the *Botanical Gazette* of 1897, last half, which he has used for finishing laboratory tables:

Solution A, 67 g. sodium chlorate.  
67 g. copper chloride.  
1 l. water.  
Solution B, 150 g. anilin chlorate.  
1 l. water.

Paint the wood with solution A, allow it to just dry, follow with solution B, and allow it to dry in. If a dense black is desired three applications will be necessary. After drying, the surface may be finished with oil as ordinary wood.

## DISCUSSION.

## Notes on Part V of Dr. Novy's Article on Laboratory Methods in Bacteriology.

The writer has followed, with interest, the articles of Dr. F. G. Novy on "Laboratory Methods in Bacteriology" in the JOURNAL OF APPLIED MICROSCOPY, but feels compelled to say that the methods for the preparation of culture media, as used in the Hygienic Laboratory of the University of Michigan, and described in Part V of Dr. Novy's article, are not based altogether on the modern theory of indicators or on the results of the latest investigations.

The writer feels compelled to take exception to Dr. Novy's method for the adjusting of the reaction of media and to his position with reference to the superiority of litmus over phenolphthalein as an indicator for use in this connection. Dr. Novy's method of titration uses up 60cc. of medium to determine the reaction of the same, and he claims no greater accuracy for it than may be easily obtained by Fuller's method (Journ. Am. Pub. Health Assn., Oct., 1895, p. 381) using only 15cc.

While criticising Dr. Novy's article, the writer has refrained from describing methods differing from his because those which the writer has accepted, in common with many others, as the most advisable have been published in the Report of the Bacteriological Committee (Journ. Am. Pub. Health Assn., January, 1898), which report was the result of joint labors of the following gentlemen: J. G. Adami, W. T. Sedgwick, G. W. Fuller, Charles Smart, A. C. Abbott, C. A. Cheesman, Theobald Smith, and Wm. H. Welch.

Dr. Novy cites the same objections against the use of phenolphthalein that Dahmen did (Centralblatt, Bd. XII, p. 260) in 1892. These objections are based on the presence in the media of ammonia salts and carbonates which may interfere with the use of phenolphthalein; they really do exist, but may be readily overcome. The amount of ammonia at the time the reaction is usually determined has been found to be less than one-tenth of the amount which interferes with the accuracy of this indicator, and the error due to carbon dioxide is obviated if the Bacteriological Committee's directions are followed. Phenolphthalein takes into account the bodies which have an amphoteric reaction to litmus, but in which the acid character nevertheless predominates. These bodies affect the growth of bacteria, while phenolphthalein is unaffected by phosphates which have no effect on the growth of bacteria.

Phenolphthalein contrasts very favorably with litmus in that the latter does not react with those bodies which affect bacterial growth, and, on the other hand, does react with phosphates which have no such effect on bacterial growth (Fuller). Phenolphthalein therefore permits the preparation of media which yield more uniform conditions of growth and thus makes quantitative bacteriological analyses more definite and reliable.

Dr. Novy seems to object to phenolphthalein because it gives a different end-point from that obtained with litmus. As far as the writer knows, no one has

ever claimed that the end-points were alike; on the contrary, the use of phenolphthalein is recommended for the reasons given in the foregoing paragraph, which would lose weight if the end-points were the same.

Phenolphthalein is not the only indicator which shows a different end-point from litmus; not only do different lots of litmus differ from one another in this respect, but all the customary indicators have different end-points according to circumstances. For example, methyl orange is not affected by carbon dioxide, because it is itself a stronger acid and therefore is not affected by the weaker acid. Phenolphthalein and turmeric are, by common consent, the best indicators for weak acids, with which latter we have to deal in media preparation. (Journ. Am. Pub. Health Assn., October, 1895, p. 389.) The following table illustrates this point, showing the reaction of various media and of their ingredients to different indicators:

Media.	Phenolphthalein.	Turmeric.	Blue litmus.	Red litmus.
* Meat infusion,	35	35	20	4
Merck's peptone,	16	16	7	2
Witte's peptone,	15	15	4	3
Sargent's peptone,	10	10	2	-1
Gelatin,	17	17	11	6
Agar,	0	0	0	-0.7
Nutrient gelatin,	56	56	31	12
Nutrient agar,	47	47	28	4

The above was condensed from an article by G. W. Fuller in Journ. Am. Pub. Health Assn., October, 1895, p. 389.

A casual inspection of the above table will show that, of the two, the litmus neutral end-point is much the more variable, and this variance is further increased because of the impossibility of making and keeping litmus paper which will give constant results. Therefore, when solutions of the character used are brought to the litmus neutral point only a partial and extremely variable neutralization is affected.

Dr. Novy concludes his comparison of the two indicators by implying that it is easier to determine the reaction of media and to duplicate the same in successive batches of media when using litmus, than when using phenolphthalein as an indicator.

The writer's experience in a number of sanitary laboratories leads him to believe that if the recommendations of the Bacteriological Committee are followed out, no difficulty is experienced, from a practical standpoint, in making different lots of media of the same reaction. It seems therefore that the evidence is easily in favor of the use of phenolphthalein as an indicator, since the reaction passes beyond accurate control when litmus and the other acid indicators are used.

In the preparation of the specific media, Dr. Novy does not specify the optimum reaction for general work. He overlooks also the advantage of having the three principal artificial media analogous in the sense of being broth, broth plus gelatin, and broth plus agar-agar.

This end is not gained best by making up broth first and then adding the requisite amounts of thread agar or sheet gelatin, but by the addition of the thread agar, or sheet gelatin, to the meat infusion at the time when the peptone and salt are added, for when thread agar or sheet gelatin and broth are boiled together for a time sufficient to dissolve the added material, the broth is heated much longer than when nutrient jellies are prepared directly. Nutrient agar can be uniformly prepared without sediment if the modern methods are followed.

Why should methods for the preparation of media be uniform? Why should different laboratories agree in their different methods? Why should the methods proposed by the Bacteriological Committee be adopted, for the present at least?

It must be evident to everyone that comparability of results is desirable, whether the work is done in one laboratory or in several, and the first two questions, therefore, need no comment. The answer to the third question, however, admits of more discussion. This question cannot be better answered than has already been done in the introduction to the committee's report (Journ. Am. Pub. Health Assn., January, 1898).

These recommendations were formulated by a committee of acknowledged eminence, and for this reason, if for no other, are more likely to be adopted than methods emanating from any single source. Apart from this, the methods recommended have, in practice, proved the most exact and probably, in the broadest sense, the simplest yet devised. The writer does not claim, any more than does the Bacteriological Committee, that these recommendations represent anything more than a basis for uniformity and for future work.

In view of the fact that so much of the data of the last ten years is in a chaotic condition, largely because of inexact methods and lack of uniformity in technique, it is to be deplored that any teacher with the reputation and standing of Dr. Novy should advocate any set of elementary methods other than those of the Bacteriological Committee which do not, at least, possess distinct advantages over those adopted by the committee.

It seems unfortunate that Dr. Novy's article, the latest on the preparation of media, should describe methods more like the empirical methods of the earlier workers in Bacteriology than those now accepted as embodying the most advanced views on these subjects.

ROBERT SPURR WESTON.

Boston, Mass., Feb. 28, 1899.

### Reply to Mr. Weston's Criticisms.

Mr. Weston feels compelled, in the foregoing "Notes," to take exception to the method of alkalization and to the other methods as described in my paper, inasmuch as they "are not based altogether on the modern theory of indicators or on the results of the latest investigations." Some doubt may be expressed as to the exact meaning of the reasons adduced. It may be said that in laboratory work practical results are sought for, and not "modern theories."

Mr. Weston is apparently troubled because of the waste of material when 60 cc. of the medium are employed to determine the reaction. I can assure him



that equally good results may be obtained by the use of 30 cc., in which case one-half of the material may be saved.

As to the different behavior of phenolphthalein, litmus, methyl orange, etc., with respect to the end-reaction, that is a fact which has been recognized in volumetric analysis for a great many years, although to Mr. Weston it may appear to be entirely modern. My objections to the use of phenolphthalein, based partly upon the interference of organic matter, ammonia salts, and carbonates, are such as are taught to a beginner in quantitative analysis. It is therefore surprising that Mr. Weston should feel compelled to go back as far as 1892, to cite Dahmen as maintaining the same objections as I do. If Mr. Weston will consult the various text-books which have been published during the past year or two, he will find that litmus is still used as an indicator, and that in many cases phenolphthalein is not even mentioned. Moreover, he will find in recent American contributions, such as those of Park and his co-workers, that litmus is employed and even preferred by some investigators. A little familiarity with the various European laboratories would likewise teach that litmus has not been superseded by phenolphthalein.

Mr. Weston also finds fault that, in the preparation of the specific media, I do not specify the optimum reaction for general work. On reference to the article in question (p. 237, 13th line from the bottom), a sentence will be found which Mr. Weston has overlooked, and which explains itself.

As to the use of the terms *broth*, *broth plus gelatin*, and *broth plus agar-agar* in place of bouillon, gelatin and agar, that is a matter of taste concerning which it is unnecessary to waste words.

Evidently, Mr. Weston has read my article in a great hurry since he infers that gelatin is added to the broth, and not to the meat infusion. It will be sufficient to refer to the 16th line from the bottom on p. 235. As to my statement that agar dissolves better in clear bouillon than in a meat extract; this Mr. Weston can readily prove by a comparative trial, but not by an *ex cathedra* denial based upon the self-complacent attitude that he employs perfect, modern methods.

Mr. Weston, furthermore, seems to greatly deplore the fact that I should employ other methods than those recommended by the Bacteriological Committee of the American Public Health Association. It is hardly necessary for me to state that I have the greatest respect for the members of that committee and for the work they have done, but as a teacher and investigator I prefer to employ methods which my experience indicates to be the best. These may seem to be empirical to one who possesses the advanced views of Mr. Weston, but nevertheless they are in accord with the practice that prevails in not a few of the best laboratories in this country and in Europe.

F. G. NOVY.

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The following test for the proteid myrosin is recommended by Gaignard, who finds it in the seeds and other parts of many of the *Crucifere*: Add one drop of a 10 per cent. aqueous solution of orcin to 1 ccm. of concentrated hydrochloric acid. Heat the sections in this solution to about 100 degrees C. A violet color will appear in the cells containing myrosin.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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permit those members of the society who are also interested in the various programmes presented by the American Association for the Advancement of Science whose meetings follow these dates immediately, to attend such as they may desire by remaining a little longer, and will also allow A. A. A. S. members to be present at the Microscopical Society's sessions by coming a little earlier. The affairs of the society are in excellent hands, Professor S. H. Gage and Dr. V. A. Moore, of Cornell University, with Dr. A. C. Mercer, of Syracuse University, constituting the executive committee; with Dr. William C. Krauss, Niagara University, president; Professor Henry B. Ward, University of Nebraska, secretary; Dr. A. M. Bleile, Ohio State University, and Dr. G. Carl Huber, University of Michigan, vice-presidents; Mr. Magnus Pflaum, treasurer. We should like to see the work of a great many laboratories represented at the coming meeting by groups of papers from the director of each laboratory, his assistants, and advanced students. The value of the information brought together, and of the discussions, demonstrations, and working sessions which could be arranged were each active laboratory thus represented, could hardly be estimated. The excellent showing made by Cornell at the Syracuse meeting is a good example of the possibilities in this line of work. Give this matter a little thought thus early and correspond with the secretary about it. On another page a blank form for application for membership in the American Microscopical Society will be found. If you are interested in maintaining the efficiency of the microscope and in improving microscopical methods, fill it out and forward to the treasurer.

\* \* \*

OUR readers will be pleased to know that Professors Alfred J. Moses and Lea Mc I. Luquer of the department of Mineralogy, Columbia University, N. Y., will conduct a department of mineralogical literature in the JOURNAL, in which reviews of the more important papers relating to mineralogy will appear. Foreign as well as American periodicals and books will be reviewed. This department will be especially welcome to those who do not have access to the numerous foreign publications.

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THE Executive Committee of the American Microscopical Society has fixed the dates of the coming meeting at Columbus, Ohio, for August 17 to 19, inclusive. This action of the committee seems very wise, as it will

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Blackman, V. H.** On the Cytological Features of Fertilization and Related Phenomena in *Pinus sylvestris*. Phil. Trans. of the Royal Soc. of London. 190: 395-426. pl. 12-14, 1898.

followed by iron-alum-hæmatoxylin and counterstained with acid fuschin also gave good results. Klein's fluid (one-sixth per cent. chromic acid 2 parts, alcohol 1 part) is also recommended on account of its excellent penetration. Material not needed for immediate use was preserved in Calbera's fluid (alcohol 1 part, glycerine 1 part, water 1 part).

This paper presents a brief but comprehensive resumé of the subject, and adds one of the most important contributions to our knowledge of fertilization which has appeared in recent years. Beginning with the formation of the ventral canal cell, the processes are traced with considerable detail up to the early stages in the formation of the sporophyte. During the great increase in size which the oosphere nucleus undergoes before fertilization, it becomes filled with a metaplasmic substance at first granular, but in later stages taking the form of a complicated network which stains deeply and might easily be mistaken for chromatin. The real chromatin consists of a few rod-shaped masses. The entire contents of the end of the pollen tube pass into the oosphere, the four nuclei being readily distinguished. As the sex nuclei come into contact, they contain numerous kinoplasmic threads which the writer interprets as the earliest indication of the first segmentation spindle. After fusion of the nuclei, the two groups of chromosomes belonging to the two sex nuclei can still be distinguished even after the first segmentation spindle is nearly formed and the chromosomes have undergone longitudinal splitting. In all other accounts of fertilization in plants, the fusion nucleus goes into a resting stage before division, the only exception being that of *Peronospora* as described by Berlese after this paper was written. From his study of the cytoplasm of the oosphere and spindle formation in the early divisions he draws the conclusion that there is no specific kinoplasmic or archoplasmic spindle forming material, but that the fibers result from a rearrangement of the ordinary cytoplasmic reticulum.

Dixon (1894) found eight chromosomes in the nucleus of the oosphere, and usually twelve, but sometimes eight or twenty-four in the large prothallial cells sheathing the oosphere. Blackman's work, which is much more thorough in regard to the chromosomes, shows twelve chromosomes in the oosphere nucleus, and also in the pollen mother cells; twelve chromosomes in the nuclei of the female prothallium and twenty-four in the sporophyte. No centrosomes were observed at any stage of the process of fertilization.

C. J. C.

Flemming's chromic-osmic-acetic followed by safranin-gentian violet-orange is the combination recommended for this work, though mercuric chloride

**Russell, F. H.** An Epidemic, Septicemic Disease Among Frogs Due to the *Bacillus Hydrophilus Fuscus*. Jour. of the American Medical Association, June 18, 1898.

Frogs have long been used to demonstrate the laws and facts of biology, and the animal has rarely shown evidences of disease; but in 1891,

Sanarelli was seriously interrupted in his work by an epidemic which broke out among the frogs in his laboratory. He found that the disease was due to a bacillus to which he gave the name mentioned in the title. Two years later a serious mortality among the frogs in the laboratory induced Trambusti to make an investigation. He found the disease to be due to a bacillus which he identified as the one described by Sanarelli. In the same year the bacillus was also identified by Rogers. In 1897 a severe epidemic, due to the same bacillus, broke out among the frogs at Rush Medical College (Chicago). The present paper, from that college, gives a review of previous work and adds a substantial contribution. The bacillus is a water organism, has one flagellum, is chromogenic, gas producing and aerobic, but also grows to some degree without oxygen. It grows upon all culture media. It is pathogenic for frogs, toads, salamanders, lizards, sunfish, and eels. It is also fatal to many warm-blooded animals. The organism produces toxins of two sorts, one resembling digitalis and the other veratrin.

C. J. C.

**Boyer, Charles S.** New species of Diatoms. Proc. Acad. Nat. Sci. of Phila. Oct. 1898.

This paper describes six new species and one new variety of diatoms, one

species belonging to the genus *Rhabdonema* and the rest to the genus *Biddulphia*. In case of one species only one specimen was found, and in another only two specimens. It would be difficult in higher plants to establish species upon so little material, especially in large genera. The descriptions are accompanied by photographs.

C. J. C.

**Ball, C. R.** An Anatomical Study of the Leaves of *Eragrostis*. Proc. Iowa Acad. Sci. 4: 1897.

It was the purpose of this study to determine whether the anatomical characters of the leaves of *Eragrostis*

are sufficiently well marked and constant to be of value in identifying the species. The writer decides that the characters are quite well marked and constant, and he accordingly devises an analytical key to the species of the genus. Several papers of the same nature have appeared in the same publication. Recent work in physiological anatomy by Warming, Grevillius, and other investigators shows how readily plant tissues vary as the environment is changed; especially is this variability characteristic of epidermal tissues. Mr. Ball's key to the species of *Eragrostis* is based on such characters as relative size of epidermal cells, length of trichomes, and number of parenchyma cells. Anyone who has made a study of plants in the field with regard to habitat variations, will recognize at once how futile it is to attempt to separate species in the laboratory by means of such anatomical characters. If this method is used at all it should be with the greatest caution and after the most exhaustive study. The anatomist of the future will not try to separate species in this artificial way, but will endeavor to bring them together by means of the intergrading forms; and the basis for this work must always be a discriminating study in the field. The attempts to



classify fossil woods on minute anatomical characters were largely set aside when it was discovered that several genera could be found growing on a single tree. Some of the artemisias are separated by pubescence characters, and it is not rare to find a single individual smooth in summer and pubescent in winter; all gradations can also be found as the habitat varies. A more careful study will surely result in our finding that many of the species of our manuals are but habitat variations. In the light of what we now know about the plasticity of plant structures, it would seem wiser to direct anatomical studies along lines that are more likely to yield profitable results.

HENRY C. COWLES, Chicago.

**Wood, D. B., M. D.** Diatoms. Trans. Vassar Bros. Inst. and its Sc. Sect. **7:** 66-86, 1894-1896.

Directions are given for collecting and mounting diatoms. They grow in either stagnant or fresh water, at any temperature, from that of polar seas to hot springs. They may be obtained by skimming off the brownish scum found on ponds, by squeezing out water weeds, by scraping sticks and stones which are covered at high water, or from the mud of the filter beds at pumping works, or in other places. The material is put in a dish of water and after it has settled the water is decanted. This is repeated until the water will clear in about one-half hour. The sediment is then treated with an equal bulk of sulphuric acid, after which bichromate of potash is added until all action ceases. After a couple of hours the acid is washed out. To separate the diatoms, place the cleaned sediment in a glass dish with water and when the water becomes clear give the dish a slight rotary motion. This will bring the diatoms to the top, when they may be removed with a pipette and placed in alcohol. To mount, place a number in distilled water, evaporate a few drops of the mixture on a cover-glass, which is then mounted on a slide in Canada balsam.

E. M. B.

**Zacharias, E.** Ergebnisse der neueren Untersuchungen über die Spermatozoiden. Bot. Zeit. **57:** 1-6, 1899.

This paper consists principally of a brief review of recent literature on the development of spermatozoids with special reference to centrosomes and blepharoplasts. In closing, he refers to the fact that recent investigators of fertilization in Angiosperms have not been able to find centrosomes, and he adds the suggestion that it is not impossible that reinvestigation may reveal the centrosomes again where they have been missed temporarily.

C. J. C.

#### RECENT LITERATURE.

**Belajeff, Wl.** Ueber die männlichen Prothallien der Wasserfarne (Hydropterides). Bot. Zeit. **55:** 141-194. pl. 8-9, 1898.

**Dittrich, G.** Zur Entwicklungsgeschichte der Helvellineen. Beiträge zur Biol. d. Pflanzen. **8:** 17-51. pl. 4-5, 1898.

**Eriksson, Jakob.** Studien über den Heckenbesenrost der Berberitze. (Puccinia Arrhenatheri. Kleb.) Beiträge zur Biol. d. Pflanzen. **8:** 1-16. pl. 1-3, 1898.

**Nelson, E. M.** On the Evolution of the Microscope. Jour. Queckett Mic. Club. **7:** 98-117. figs. 10-23, 1898.

**Reed, J. W.** The Aecidium stage of Uromyces pisi. Jour. Queckett Mic. Club. **7:** 65-74, 1898.

**Saunders, De A.** Phycological Memoirs. Proc. Calif. Acad. Sci. [Contributions to Biology from the Hopkins Seaside Laboratory of Leland Stanford, Jr., University XVI.] Proc. 3rd. Ser. Botany **1:** pp. 147-168. pl. 12-30, 1898.

## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
 Agnes M. Claypole, Sage College,  
 Ithaca, N. Y.

## CURRENT LITERATURE.

**Newbigin, M. S.** On certain Green Pigments  
 in Invertebrates. Quart. Journ. Micro. Sci.  
 N. S., 163: pp. 391-431, pl. 30, 31.

This investigation was started and  
 carried on to decide, if possible, the  
 nature of some of the green pigments

found in invertebrates and hitherto considered as closely allied to or identical with plant chlorophyl. A pigment occurring largely in the alimentary tract and digestive glands has been called entero-chlorophyl to show both its similarity to and difference from plant chlorophyl. Chaetopterin and bonellin are other well known invertebrate pigments found by the author in these forms. Without going into details, it may be said that the animal used principally for this study was the common limpet (*Patella vulgata*), and entero-chlorophyl was obtained by drying the tissues for chemical and spectroscopic studies: liver, intestines, and the contents of the gut being all used. Histological preparations were made from tissue prepared in formalin, as this fixer did not destroy the pigment granules, which event occurred with ordinary hardeners.

Most of the investigation was microspectroscopic with solutions of the pigments in alcohol, changes on treatment with acids, alkalies, and certain salts being carefully described. A most careful examination has been made and the spectra figured. The author concludes that there exists in invertebrates a widely spread group of pigments that occur primarily in the alimentary canal or its outgrowths. These pigments form with alcohol solutions characteristically fluorescent, of an indefinite color and having a spectrum of five bands when fully developed. In complexity of spectrum and fluorescence they resemble chlorophyl, but not in chemical reactions to acids and alkalies. Whatever their primary function, they resemble the bile pigments of vertebrates in occurring with the contents of the gut and in their elimination with the fæces. A very interesting point came out in the tests conducted to demonstrate the essential difference between entero-chlorophyl and chlorophyl. On treatment with certain acids a brilliant green color with a peculiar fluorescence is obtained. This can be destroyed by the addition of alkalies, but will again return on treatment with acids, thus showing a greater stability of structure in entero-chlorophyl than in plant chlorophyl, which readily decomposes on similar treatment and does not show any power to return to its former state.

The author concludes that entero-chlorophyl is a substance of simpler construction than plant chlorophyl, a conclusion in harmony with the general facts as to animal substances.

A. M. C.

**Bensley, R. R.** The Structure of Mammalian  
 Gastric Glands. Quart. Journ. Micro. Sci.  
 163: pp. 361-390, pl. 29.

The author has taken up the question  
 of the difference between the pyloric  
 and peptic glands of the stomach, and

finds that the views of Heidenhain, Ebstein, and Grützner are not longer tenable.

These older views maintained that the chief cells of the peptic glands were similar to the chief cells of the pyloric glands, the only difference in the two kinds of glands being the absence of parietal cells from the pyloric glands. After a careful historical review of the subject which shows that differences in the cells from different parts of the fundus glands had already been noted, the author gives the results of his studies on the rabbit, dog, and cat, especially the last two animals.

The method employed was to cut off bits of gastric mucosa with scissors directly into Foa's blood-fixing fluid (equal parts of mercuric chloride in 95 per cent. alcohol and a 2-4 per cent. aqueous solution of potassium bichromate), leaving them there from one-half to two hours, according to size. Then they were washed in 70 per cent. alcohol for twenty-four hours, or until all the bichromate was removed. They are then put into 95 per cent. alcohol and imbedded in paraffin by the oil of bergamot method. Control specimens were carried through in alcohol, aqueous bichromate mixtures, and Hermann & vom Rath's osmic mixtures. The Foa mixture was found to fix the granules perfectly.

Biondi tricolor stain, Mayer's hemalum, Erlich's acid hæmatoxylin, and many other special stains were used, among them stains to show the presence of iron compounds in the cell.

The author concludes that in the cat and dog the fundus or peptic glands contain two kinds of chief cells, those of the body and those of the neck of the gland. The former secrete ferment, as shown by the presence of zymogen granules near the lumen. There is also in the outer part of the cell a deeply staining substance of the nature of chromatin, which stands in a genetic relation to zymogen and is known as prezymogen. The neck cells are considered to be the same as the pyloric gland cells in the cat and dog because neither kind of cells contains zymogen granules and only traces of prezymogen. Both groups secrete a substance reacting similarly to certain stains that may be called mucin. Mucin cells have been also found in the fundus glands of the mink, rabbit, mouse, rat, squirrel, ground hog, chipmunk, pig, and sheep. The further relations of the glands will be discussed in another paper.

A. M. C.

**Morgan, T. H.** Experimental Studies of the Regeneration of *Planaria maculata*. Archiv. f. Entw. der Organismus. 7: pp. 364-397, 41 figs. in text, 1898.

For convenience the author describes experiments under the following headings: (1) Regeneration of cross-pieces from various parts of the body. (2)

Regeneration of short pieces from the side. (3) Regeneration of small pieces from the posterior end. (4) Regeneration of long pieces from the side. (5) Regeneration of triangular pieces. (6) Regeneration of very short cross-pieces. (7) Regeneration of a head at the posterior end of a cross-piece. (8) Transformation of an old piece into a new worm.

Material was collected in the vicinity of Wood's Holl, Mass., and the experiments continued from June during the following winter. The largest of the worms used measured about 20 mm. in length and one-half mm. in breadth. Most careful experiments were carried out, and the following gives in part a summary of his results. Cross-pieces of the body develop a new head and a new tail, the

new tissue merely forming the head and tail, all growth taking place in the old tissue. If the cross-piece is near the head of the animal, the pharynx, which is normally placed nearly centrally, develops in the posterior part and between the old and new tissue; if the piece is from the middle of the animal, it appears near the middle of the piece. The anterior end of the body in front of the eyes will not form a new worm, but if the piece merely includes the eyes such a growth takes place. A great range of regenerative power was shown in pieces of various shapes and sizes. Position of the piece seemed more important than size, as small pieces from certain places grew while larger from others died. In one case a head developed at each end of a piece, and sections showed a distinct double nervous system. No efforts to produce such a growth succeeded, so the cause for the one case is unknown. New vegetative organs may appear in any part of the tissue, but the new eyes and brain only develop when the new part first appears. In conclusion, the author finds that the material of the body is almost as plastic as that of a developing egg.

**Müller, E. Drüsenstudien.** Zeitschr. f. wiss. Zool. 64: pp. 624-647, 2 pls.

The author used other means than the famous Golgi method to demonstrate the secreting capillaries of the gastric glands found in the stomach of a dog. The tissue was laid for twenty-four hours in a mixture of 40 pts. 3.5 per cent. potassium bichromate and 10 pts. of commercial formalin), and then for one or more days in bichromate alone. They were then washed and hardened in alcohols, increasing strengths. The sections were stained in Heidenhain's iron hematoxylin and gave admirable results, serving well to confirm those of the Golgi process. The author shows the existence of not only the usual cross channels to the lumen, but also the intracellular secreting capillaries. The fluids of both central and parietal cells rise from granules which are at first stainable and later lose this power. In the parietal cells only are there intracellular canals. The author shows from further work on the secreting glands of the cat that empty mucous and serous cells cannot be distinguished from each other nor from Gianuzzi's crescents, but their distinction depends on the cell contents. He further urges that more consideration be paid to the way in which cell contents change from a granular to a fluid condition.

**Nicholls, A. G.** Sudan III, a Selective Stain for Fat. Microscopical Bulletin, 15: p. 31.

The author in this note confirms the work of Rieder in finding this stain, Sudan III, useful in histological work, especially in staining fat. A saturated solution of Sudan III in 96 per cent. alcohol is filtered, and diluted two-thirds with 50 per cent. alcohol, and again filtered. Sections are allowed to stay in this mixture for a few minutes and then washed in 60 to 70 per cent. alcohol, drained and mounted in glycerine or Farrant's medium. Fat takes a carmine red stain, appearing golden yellow in small particles.

**Dendy, A.** Development of Sphenodon. Proceedings of the Royal Society, London. 63: pp. 440-443.

The author summarizes his results in the study of Sphenodon and finds the following important features. The whole period of development lasts thirteen months. Eggs are laid in November and hatched in December of the next year. As is frequent in such cases, the



earlier stages are rapidly passed and practically no change takes place during the winter. The formation of the amnion shows a distinct posterior amniotic canal similar to that of chelonians. A parietal eye rises distinct from the epiphysis and the pineal gland is probably represented by a mass of convoluted tubules lying in front of the parietal eye. *Lacerta* and *Anguis* have been considered by Béraneck to show a similar independence of this eye.

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- Ziegler, H. E.** Experimentelle Studien über die Zelltheilung. Pt. III, Beroë ovata. Archiv. f. Entwick. der Organismen. Bd. **7:** pt. 1, pp. 34-64, 1898.
- Rhumbler, L.** Physikalische Analyse von Lebenserscheinungen der Zelle. I. Bewegung, Nahrungsaufnahme, Defäkation, Vacuolen-Pulsation und Gehäusebau bei lobosen Phizopoden. Archiv. f. Entwick. der Organismen. Bd **7:** pt. 1, pp. 104-198. Taf. VI-VII, figs. 1-42 in text.
- Harrison, R. G.** The Growth and Regeneration of the Tail of the Frog Larva. Archiv. f. Entwick. der Organismen. Bd. **7:** pt. 1, pp. 430-481. Pts. X-XI, 21 text-figs.
- Bristol, C. S.** The Metamerism of Nephelis. A contribution to the Morphology of the Nervous System, with a Description of Nephelis lateralis. Journal of Morph. **15:** No. 1, pp. 17-72, pl. IV-VIII.
- Gardiaer, E. G.** The growth of the Ovum. Formation of the Polar Bodies and the Fertilization in *Polychoerus candatus*. Journ. of Morph. **15:** No. 1, pp. 73-110, pls. IX-XII.
- List, R.** Beiträge zur Chemie der Zelle und Gewebe. I. Ueber die Farbung tierische Gewebe mit Berlinblau. Mittheil d. Zool. Station Neapel. **12:** p. 477, 1896.
- Neigert, C.** Ueber eine Methode zur Farbung elastischer Fasern. Centralbl. f. allgem. **289** Pathol. u. Pathol. Anat. **9:** No. 8, 9, p. 2, 1898.
- Comte, L.** Contribution à l'étude de l'hypophyse humaine et de ces relations avec le cerps thyroïde. Beitr. z. Pathol. Anat. u. Allgem. Pathol. **23:** No. 1, p. 90, 1898.

### CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review  
to H. H. Waite, 710 East Catherine street,  
Ann Arbor, Michigan.

**Caselli, A.** Experimentelle und bacteriologische Untersuchungen über das Puerperalfieber. Centralbl. f. Bakt. **25:** 5-10, 1899.

This article contains a series of investigations attempting to establish the relationship of pus organisms to puerperal fever. Caselli's experiments were made on rabbits and he divides them into four classes:

1. Effect of the introduction into the empty uterus, whose walls have been injured, of the streptococcus erysipelatis.
2. Effect of the introduction into the pregnant uterus, whose walls have been injured, of the streptococcus erysipelatis.
3. Effects of tampons infected with streptococci when introduced into the injured vagina of pregnant rabbits.
4. Effect of infected tampons on the normal vagina of pregnant rabbits.

Of the animals experimented on in the first series all died in from one to three days. Streptococcus infection was established in every case through bacteriological examination of sections prepared from organs and tissues taken

from different parts of the body. This series shows also the virulence of the streptococci and their capability of causing the death of the not pregnant animal through spreading, by continuity, to other parts from the uterus.

In the second series, it was shown that the streptococcus infection first brought on abortion and later caused the death of the animal.

In the third series, four rabbits were inoculated with the streptococcus and all died in from three to eight days after inoculation. In sections from the organs were found extensive changes as a result of the infection.

The streptococcus, as this series shows, can produce a rapidly fatal infection by spreading from the mucous membrane of the vagina to other parts.

The fourth series of investigation is the most interesting inasmuch as the animals operated on show that the streptococcus, introduced into the normal vagina, may remain virulent for a long time, even causing severe infection, at the delivery of offspring, occurring forty-five days after the introduction of the streptococcus into the uninjured vagina. The disease caused by this infection may appear in different forms; at one time as an acute general infection; at another, in the form of abscesses of the pelvis, perioöphoritis, salpingitis, or embolic suppuration in remote organs. The most important result obtained from these investigations is the fact that the streptococci may remain in the vagina for a long period of time, maintain their virulence, and, as was shown by the experiment, be capable of causing disease forty-five days after their introduction. They remained dormant until a lesion was formed through parturition, but as soon as this was furnished infection took place.

H. H. W.

**Kanthack, A. A.** Influence of the Milk  
**Sladen, E. S. St. B.** Supply on the Spread of  
Tuberculosis. *The Lancet*. 74-78, 1899.

This work covers the examination of milk from sixteen dairies supplying Cambridge University. They found the milk received from nine, or 60 per cent., was tuberculous. Of ninety guinea pigs inoculated with milk from all these dairies twenty-three died, or 25.55 per cent. Some were inoculated with the cream obtained by centrifugation, and others with the sediment at the bottom of the centrifugal tube. Of those that died, thirteen were inoculated with the cream, and ten with the sediment. In sixteen of the twenty-three animals that died the tubercle bacillus was demonstrated in the organs of the dead animals.

H. H. W.

**Seybold, C.** Ueber die desinficirende Wirkung des Metacresols Hauff im Vergleich zu Orthocresol, Pacacresol, Tricresol Schering, Phenol und Guajakol. *Zeitsch. f. Hyg.* 29: 377-418, 1898.

Seybold found from his experiments that metacresol, orthocresol, paracresol, tricresol, phenol, and guaiacol, in two per cent. solution, are equally ineffective and do not kill anthrax spores even after an exposure of twenty-six days. From his tables it is shown that the cresols possess a greater germicidal effect against the vegetative microorganisms, staphylococcus pyrogenes aureus, bacillus pyocyaneus, and bacillus prodigiosus, than do phenol and guaiacol. Among the cresols the metacresol of Hauff is the most effective, paracresol comes next, orthocresol and tricresol act equally well and are the weakest. Paracresol proved to be the most poisonous, and metacresol the least poisonous,

of the isomeric cresols. By the addition of 18 grams of sodium chloride to 100 cc. of a half per cent. metacresol solution, the disinfecting power is considerably increased. For common use this large amount of salt is not practicable. A smaller amount, however, has no effect. Seybold advises the use of Hauff's metacresol for the following reasons: 1. Metacresol has a considerably greater disinfecting power than has phenol. 2. Metacresol is not as poisonous as phenol. 3. A two per cent. water solution is clear and does not have an injurious action on the hands or on instruments, and the solution has very little odor.

H. H. W.

## NEWS AND NOTES.

A HOME-MADE DARK ROOM WINDOW.—We have recently experimented on coloring ordinary glass so that it may be used in place of ruby glass in the photographic dark-room of the Biological Laboratory, University of the Pacific. A pane of ordinary window glass was coated on one side with a solution of gelatin colored with erythrosin. The colored gelatin was allowed to run on the glass as evenly as possible and dried. The erythrosin absorbs all the green rays, but permits the violet rays to pass through. It is therefore necessary to coat the opposite side of the glass with gelatin, colored with orange G, which absorbs the blue and violet light. The gelatin surfaces were coated with varnish to protect them, and the glass thus prepared forms an excellent light for developing plates by the daylight.

RUE D. FISH.

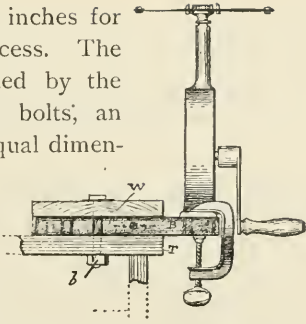
HARDENING AND IMBEDDING DELICATE OBJECTS.—For some time I have made use of the following method for hardening and imbedding fragile and delicate tissues and specimens. A piece of thin muslin or cheesecloth is arranged in the form of a sack having a capacity three or four times as large as the volume of the specimen. The object to be hardened is placed in the sack and the whole may be suspended in the hardening fluid, or placed on the bottom of the vessel. By taking hold of one side of the sack the object may be shifted without danger of injury. I have found this method useful in hardening the brain of such animals as the sparrow, cat, and sheep. In hardening histological material the tissues are in no danger of being confused or lost if each sack is numbered and recorded. I have in some cases carried the tissue up to imbedding in paraffin very conveniently.

E. G. BURCH.

Fargo, North Dakota, High School.

HOW TO OBIVATE THE VIBRATION OF CENTRIFUGES.—A few days ago I changed electric power, the speed of which is uncertain, for hand power. When I attempted to use the hematokrit in my office at anything approaching an efficient speed, the vibrations were so violent as to disturb the occupants of the building. The custodian demanded to know if I was running an air-ship. Every piece of furniture, to which the machine was attachable, was tried in vain and as I needed an estimation at once, the apparatus was placed upon a folded towel and held by an assistant. This answered fairly well. I then clamped the

book into a copy press, leaving a projection of two inches for the attachment of the centrifuge, with perfect success. The next step in the process of evolution is illustrated by the accompanying diagram. Two three-inch carriage bolts, an old book, 20 x 14 x 2 cm., and a block of wood of equal dimensions, are the requisites. These are fastened to the edge of the work-table T, the book B projecting 6 cm., the block W being clamped transversely over the book with the bolts B, which come on each side of the latter. Washers under the heads of the bolts insure greater stability. By this arrangement the urine attachment cannot be heard in the next room, and the sound from the hematokrit about equals that of an electric fan.



Instructor in Pathology, etc., Memphis Hospital Medical College.

WM. KRAUSS, M. D.

*Editor Journal of Applied Microscopy:*

HARTFORD, CONN.

DEAR SIR: In the next number of the JOURNAL, kindly give a list of works which give the best methods for mounting, treating, etc., different objects—a series of directions such as Dodge gives in outline in his *Elementary Biology*. Please name also the best work on the Microscope and Botany, giving technical instruction, and oblige,

A SUBSCRIBER.

**HELPFUL BOOKS IN MICROSCOPIC BOTANY.**—The following brief list of books, prepared at the request of the editor, makes no pretense to being complete, but is thought to contain the titles and sufficiently clear descriptions of several manuals which will be of material assistance to those amateur workers in microscopic botany who have not had an opportunity to receive laboratory instruction:

**Strasburger.** *Handbook of Practical Botany for the Botanical Laboratory and Private Student*, translated by W. Hillhouse, New York, published by Macmillan; \$2.50, 2d edition, revised 1897, 425 pp. 116 figs.

The character of this indispensable work is best shown by extracts from the author's preface: "This book is intended chiefly for those who, without

desiring to become botanists by profession, wish nevertheless to become acquainted with the elements of scientific structural botany. It will likewise introduce the beginner to the various methods of microscopical manipulation." "The objects for study have been so selected that most can be obtained with comparative ease." "The list of necessary reagents will be found at the end of the book." "The method of preparation of special reagents for histological work is also given in this list." "The explanations and illustrations of the use of the instruments and reagents are scattered in the text; but the general index is made so far complete as to enable the student easily to refer to any explanations which may be necessary." "All the figures in this work have been drawn from nature. Almost all of the facts given in the text, even those which were well known, have been submitted to careful control. At the close of each chapter are given some bibliographical notes, which show the student the fountain-head whence fuller information can be obtained." The manual is divided into thirty-two chapters including among other topics treated the use of the microscope, cell contents, movements of protoplasm, the various plant tissues, fibro-



vascular bundles. structure of foliage and floral-leaves, vegetative organs of mosses, liverworts, fungi, lichens and algæ, the reproductive organs of the same, cell division and nuclear division, etc. This work is by far the most valuable manual for the independent worker in microscopic branches of botanical work.

**Bower and Vines.** A Course of Practical Instruction in Botany. London, Macmillan, 370 pp. 1885. \$2.25 (?).

microchemical reagents, general structure of the cell and its microchemical and microphysical properties, studies of certain angiosperms, gymnosperms, pteridophytes, mosses, algæ and fungi. The material needed is easily obtained and the directions for work are very clear.

**Arthur, Barnes, and Coulter.** Hand-book of Plant Dissection. Holt & Co., New York, 1893. 256 pp. 2 plates; \$1.20.

(*Protococcus*), oscillaria, pond scum (*Spirogyra*), white rust (*Cystopus*), lilac mildew, liverwort, moss, maiden-hair fern, Scotch pine, field oats, trillium, and shepherd's purse. Both gross and microscopic anatomy are studied. References to standard literature assist the student to extend his investigations.

**Clark.** Practical Methods in Microscopy. Boston: Heath & Co., 1896. 261 pp. 52 wood-cuts, 8 plates of photomicrographs; \$1.60.

Is an excellent manual for the independent student. It contains directions for preparing specimens for study,

Contains directions for obtaining, preserving, and studying a well-selected series of plants, including green slime

While this is a work on general microscopic methods, it contains several chapters of interest to the botanist.

**Zimmerman.** Botanical Microtechnique, a handbook of methods for the preparation, staining, and microscopical investigation of vegetable structures; translated by J. E. Humphrey. New York: Holt & Co., 1893. 296 pp., 63 figures, \$2.50.

This work consists of a description of methods of investigating the various component parts of cells and the products of cellular activities in plants.

CHARLES WRIGHT DODGE.

University of Rochester.

**CEMENTS FOR FLUID MOUNTS.**—In the Journal of the Oreckett Microscopical Club, Mr. Rousselet once more calls attention to that never-failing source of trouble among microscopists—the failure of cements enclosing mounts in watery and other media. Mr. Rousselet finds that the varnish sold as “Spirit-proof Cement,” while answering well for media containing alcohol, fails completely to effectually seal non-alcoholic preservative fluids. It would appear that gold size, or cements having this substance as their basis, must be regarded as the most hopeful. It is rather suggestive that a series of slides which have stood the test of many years uninjured were mounted in the first instance with a small bubble of air purposely enclosed in each with the object of allowing for expansion consequent on variations of temperature. Most of the slides mounted in such cells, that we have seen, from the Jersey Biological Station, contain bubbles of air, but we are not aware whether these were included purposely or not. If so the plan can hardly be considered so satisfactory in its working with the Jersey mounts, as in several in our possession the bubbles have grown so large as to seriously interfere with the objects.

G. H. BRYAN.

# Journal of Applied Microscopy.

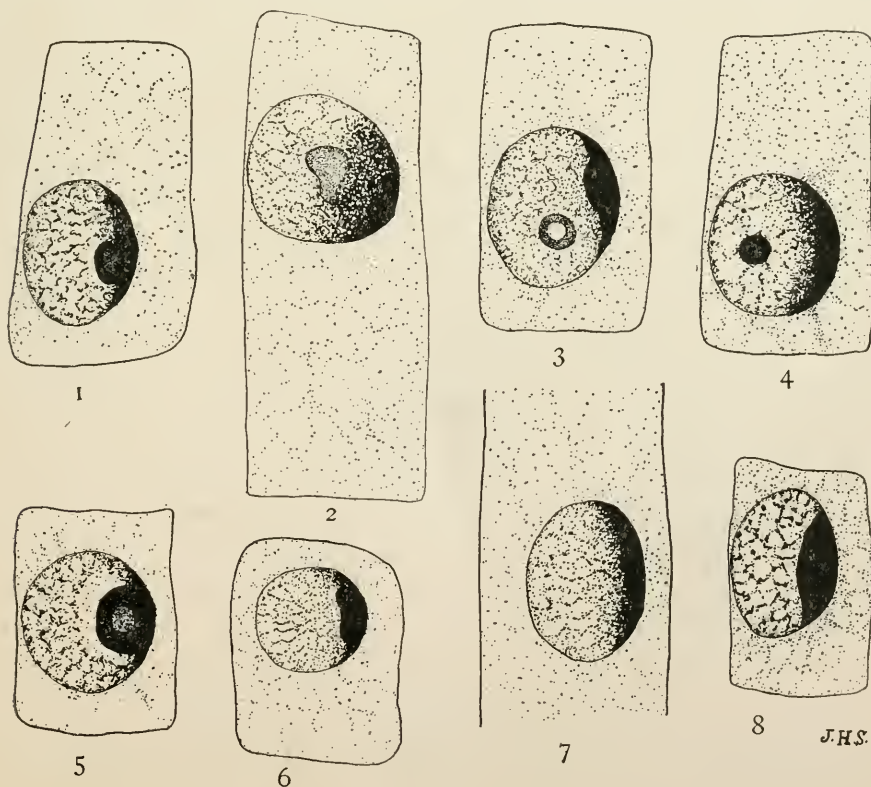
VOLUME II.

APRIL, 1899.

NUMBER 4

## Artificial Production of the Sickie Stage of the Nucleolus.

In most cytological studies the important question of correct interpretation is constantly present, and great care must often be exercised in determining whether the various appearances one meets are natural, or whether they have been produced artificially during the process of preparation and mounting, according to the methods at present in vogue. One of these appearances, which has received various interpretations, is the so-called sickie stage of the nucleolus, which



Zimmermann regards as a normal process during certain nuclear divisions. Humphrey, however, considered Zimmermann's sickle stage as an artificial production, and this seems also to be Strasburger's view.

While experimenting on the action of various killing fluids, the writer made up the following mixture, which was tried on some root-tips of the common onion :

Absolute Alcohol,	. . . . .	95 cc.
Chloroform,	. . . . .	5 cc.
Glacial Acetic Acid,	. . . . .	1 cc.
Chromic Acid (1 per cent. aqueous solution),	. . . . .	1 cc.

This fluid had an exceedingly violent action on all cell structures. The cells were badly shrunk, the nuclei displaced, and the cytoplasm more or less distorted.\* Nearly all the nuclei in the cells of the peripheral layers showed the sickle stage. In the central cells there was little displacement, although the cells were much shrunk. This was probably because the killing fluid, penetrating from opposite sides, met here, and thus had an equalizing effect. These nuclei were nearly all in the resting stage, as will appear from an examination of the figures. The nuclei were nearly all crowded toward the peripheral walls of the cells, while the nucleoli were generally pushed in the opposite direction toward the inner sides of the nuclei. No such displacement of nuclei is to be seen in well-killed sections. In figures 5, 6, and 8 are shown nucleoli in various stages of distortion. Figure 5 shows the nucleolus merely crowded against the nuclear membrane, while in figures 6 and 8 it is considerably flattened. In figure 7 the dark, sickle-shaped layer on the inner side of the nucleus is probably due to a distortion of the nucleolus with some chromatin deposited on this. Although the chromatin was usually in the resting condition, it was brought into a partial synapsis stage by the violent action of the fluid. Figures 1 and 2 give the general appearance where the dark layer of material is apparently produced by a contraction of the chromatin on the inner side. In figures 3 and 4 the sickle-shaped body is caused by a distorted nucleolus around which is a layer of chromatin.

The writer has studied the cells of the onion root-tip for years, but has never observed the sickle stage in them before. If such characteristic figures can be produced in resting nuclei in wholesale quantities, it does not seem that much importance should be attached either to the sickle stage of the nucleolus or to the one-sided contractions of the chromatin which appear in the prophase of karyokinesis, when the chromatin band is orienting itself freely inside of the nuclear membrane, and when the nucleoli are lying free in the achromatin of the nuclear cavity.

JOHN H. SCHAFFNER.

Botanical Laboratory, Ohio State University.

## The Use of Acetone in Histology.

*Preliminary Note.*—Acetone, although not officially recognized by the United States Pharmacopœia, is, nevertheless, used to a greater or less extent in therapeutics and for technical purposes.

It is stated in the dispensaries that acetone is produced by the dry distillation of woods, acetates, gums, certain carbohydrates, etc., and has a specific gravity ranging from 0.7920 to 0.8008. It boils at from 56 degrees to 58 degrees C. It has an empirical formula of  $C_3H_6O$ , although constitutionally it is Dimethyl-ketone,  $CO, CH_3, CH_3$ . It is soluble in all proportions in water, alcohol, ether, chloroform, and volatile oils, and dissolves pyroxylin, camphor, fats, and many resins.

Its action upon pyroxylin, with which this note has chiefly to deal, seems to be more intense than the customary ether-alcohol mixture. It dissolves not only the soluble-cotton form of pyroxylin, composed of the lower nitrates of cellulose, but the true gun cotton or hexanitate of cellulose, which the ether-alcohol mixture cannot do.

The collodion process has attained such an importance in histological technique, that any modification in the way of abridging some of the numerous steps, provided the good results are not interfered with, should be worthy of note.

*Fixative Action.*—Acetone also possesses desirable qualities as a fixing, hardening, and dehydrating agent, retaining to some extent the natural color of some of the tissues; so that a piece of tissue hardened in it may be placed in a weak solution (4 per cent.) of acetone collodion, and then in a stronger solution (8 per cent.), mounted upon a block and immersed in chloroform; or if some other fixing agent has been used the tissue may be placed in acetone to complete the hardening and to prepare it for the collodion baths.

In addition to the fixing and dehydrating properties of acetone, it seems also to be useful as a solvent for many of the dyes used in histology. Further experimentation is, however, necessary. Another feature, which may be of some importance, is that the pyroxylin is very readily soluble in a mixture of equal parts of acetone and ether, and the official collodion is also freely miscible with the acetone collodion.

A table comparing the acetone and alcohol methods is herewith appended. If the fixing agent has been of an aqueous character, the process is lengthened.

	6 per cent. Collodion (official).
	↑
8 per cent. Acetone Collodion.	3 per cent. Collodion (official).
↑	↑
4 per cent. Acetone Collodion.	Ether-alcohol.
↑	↑
Dehydration	Dehydration
(strong acetone).	(strong alcohol).
↑	↑
Fixing Agent	Fixing Agent
(acetone 70 per cent).	(alcohol 70 per cent).

(To be read from below upwards.)

In the commercial acetone a small amount of water or alcohol may exist as impurities, and if this grade of acetone be used in making the collodion, the collodion may become opaque and require a longer time to clear when placed in



the chloroform. Not infrequently the chloroform itself is at fault, as it may also contain a slight amount of water. This difficulty is obviated or lessened by using pure acetone and pure chloroform. Those using the oil method of cutting collodion sections may abridge the process still further by omitting the use of the chloroform and immersing directly in the clarifying agent.

*Incompatibility.*—The question of incompatibility with the fixing agents ordinarily used is of considerable importance, but the fact must be kept well in mind that an incompatibility with a *solution* of the fixing agent when acetone is mixed with it, may be of quite a different character than when the *tissue*, hardened in the fixing agent, is brought in contact with acetone.

There is always some reaction between the protoplasm of the tissue and the fixer; the formation of various albuminates, etc.; as for example, when an equal amount, or excess, of acetone be added to a solution of potassium dichromate, a slight precipitate is formed, but not nearly equal, in amount, to the precipitate thrown down when alcohol is added to a solution of dichromate. If, on the other hand, a piece of tissue hardened in the dichromate be immersed in acetone, there is no perceptible precipitate for a long time, although the acetone takes on a yellowish tinge from the salt. The range of incompatibility, so far as determined, is decidedly less than that of alcohol.

*Cost.*—The cost of the chemically pure acetone averages from eighty to ninety cents per pound. Many specimens of the commercial acetone, however, seem to be quite free from water and to answer the purpose just as well as the chemically pure for histological uses, with the further advantage that its cost is only thirty or thirty-five cents per pound; and still lower rates may be obtained if bought in larger quantities.

To private workers, especially, who are not able to avail themselves of the facilities of college laboratories, the use of acetone would seem to be of considerable benefit. It is easy to procure; it is much cheaper than the usual ether-alcohol mixture, and shortens the process.

Acetone should not be confounded with aceton,—the latter being a proprietary grip and headache remedy.

PIERRE A. FISH, D. SC., D. V. S.

New York State Veterinary College, Ithaca, N. Y.

## Demonstration of Karyokinesis.

In dealing with large classes, the satisfactory illustration of the process of karyokinesis is not easy owing to the difficulty in obtaining material which has the elements of the mitotic figure large enough to be made out with the ordinary student microscope, and which is undergoing division rapidly enough to show many different phases of the process within a single section or a limited number of sections. In animal tissues these conditions are best met perhaps by the testis of the cray-fish or lobster taken at the breeding season. By reason of the large size of the chromosomes, certain plant tissues also, such as the growing tip of the onion, have become favorite demonstration objects with biologists. Excellent for the purpose as these objects are, they are nevertheless much

inferior to the root-tips of the common Podophyllum or May-apple. In this plant the chromosomes are very large and in sections stand out like ropes, the mitotic figures are abundant in the growing root-tips, and the material is easily obtained.

An object so exceedingly favorable for the purpose deserves to be generally used by teachers of biology, though, so far as I am aware, it is almost unknown among the zoölogists. It was recommended to me by Professor D. T. MacDougall, and has done such excellent service in my classes that I believe other biologists will find it a welcome addition to their list of available class material.

The following simple method of preparation has given satisfactory results in my experience: the root-tops, freshly pulled from the moss in which they were growing, were cut off and dropped at once into Hermann's fluid. After washing and dehydrating they were stained *in toto* in Kleinenberg's hæmatoxylin and embedded in paraffine for sectioning. Sections about  $7\mu$  thick, cut parallel with the long axis of the rootlet, are most satisfactory. Thinner sections than these do not show entire mitotic figures. Under a Bausch & Lomb  $\frac{1}{6}$ -inch or a Leitz No. 7 objective, spirem stages, chromosomes, and achromatic spindles may be made out with a clearness equaled by few objects under a  $\frac{1}{12}$ -inch oil-immersion.

W. S. NICKERSON.

Laboratory of Histology and Embryology, University of Minnesota.

## Collodion Sectioning of Golgi Preparations.

In cutting sections of nervous tissue impregnated by the Cox-Golgi method, I have adopted a method which I have never seen described, but which may be employed by others. After the tissue has been impregnated in the usual manner, it is rinsed off with water, and is then placed for not over half an hour in each 95 per cent. and absolute alcohols, and for the same length of time in a mixture of equal parts of absolute alcohol and ether. Next comes immersion, for say twenty minutes, in thin and then in thick collodion, and then it is imbedded as usual. So far the process is the usual one. To harden the collodion I employ chloroform instead of weaker grades of alcohol, not only on account of its more rapid action, but because its effects are less injurious. As soon as the collodion has become somewhat firm, the paper employed in imbedding is removed and the block is placed in a mixture of one part carbohc acid and three parts xylol. In this it can be kept for weeks in a good condition. Indeed I have cut sections which showed fairly good pictures after a stay in the xylol-carbohc mixture of two years. The sections are cut with a razor flooded with the xylol-carbohc fluid, and are at once transferred to the slides and mounted directly in balsam. This method, as will be seen, has several advantages; its greatest drawback is the disagreeable effect of the carbohc acid, which is almost certain to get on the hands, no matter how carefully one may try to avoid it. I may say, in closing, that I use photographers' gun cotton instead of celloidin in making my collodion. I think it works better; it is certainly much cheaper.

J. S. KINGSLEY.

Tufts College.

## Biological Tables.

There is great uniformity in style, height, and general make-up of chemical tables, at least in the main plan. There are the shelves for bottles, the sinks, drawers, space below with door, gas, and water. But in tables devoted to work in biology there is great difference in styles and in the opinions held in regard to the efficiency of the different styles and makes. While planning the outfit for the work in biology in the new buildings of the University of Montana, it was desired to have tables that would embody the best ideas in material, structure, and finish, considering that expense was an important item. With this idea, correspondence was held with a number of professors in different American institutions, some of the plans and correspondence being here given.

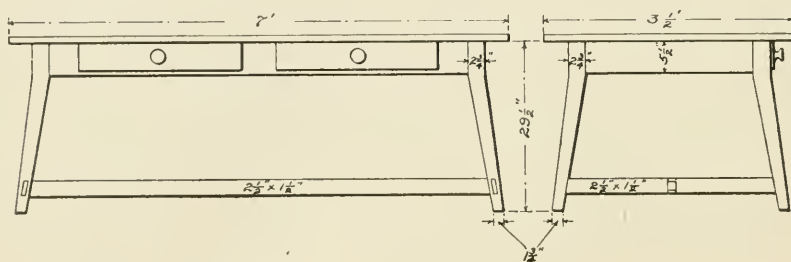


Fig. 1. Side and end view of table at Leland Stanford University.

Tables for biology are of the following patterns: for individual students; for several students at the same table; high enough to work standing or with revolving stool; high enough to use a microscope vertically while sitting on a common chair; tops of wood; tops of slate or other similar material. Most of the tables the writer has seen or read of combine the above features in varying ways, making tables to suit individual ideas or special work to be done. At first sight it would appear that any table is good enough to work at with a microscope and

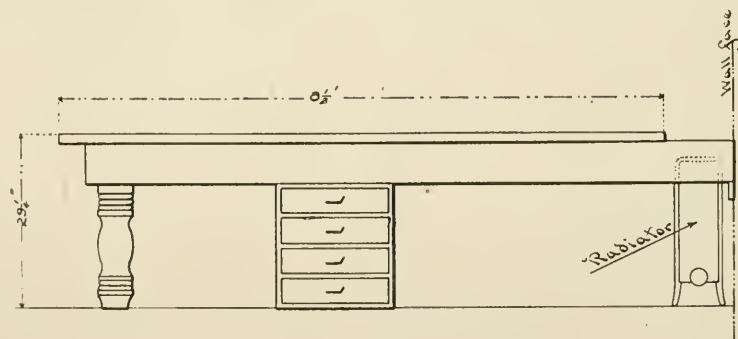


Fig. 2. Side view of table at University of Rochester.

reagents, with a drawer for storing a few utensils and some glassware. Indeed, it would appear that in some institutions very little thought is given to the tables or to the comfort and convenience of the student while at work. There is cer-

tainly as much reason for planning a table suited for the average work in biology as for work in chemistry. However, as the work in biology is so different in different institutions, while that in chemistry is in the main quite uniform, it is not surprising that opinions in regard to tables should be so varied.

First, in regard to height. The height of tables at Leland Stanford, Fig. 1, is 29½ inches; at the University of Rochester, Fig. 2, 29¼ inches; at University of Iowa, Fig. 3, 42 inches; at University of Illinois, Figs. 4, 5, 6, 30 inches; at University of Montana, Fig. 8, as planned by the writer, 28 inches; at Cornell University, as shown in the JOURNAL OF APPLIED MICROSCOPY for February, 1898, 28½ inches. All the tables mentioned certainly consider that the student should sit rather than stand. Indeed, for the most work in different lines of biology the student should certainly sit. From a physiological standpoint it is

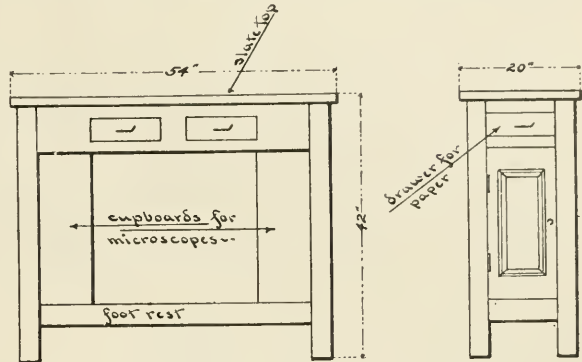


Fig. 3. Side and end view of table at University of Iowa.

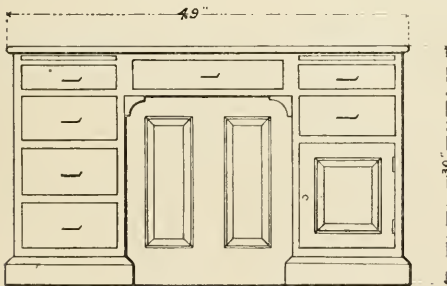


Fig. 4. a. Side view, table at University of Illinois.

better to sit on an ordinary chair, with the feet resting comfortably on the floor. Sitting thus, a table top 28 or 29 inches high gives greatest comfort for work with vertical microscope. A table of this height is not comfortable or convenient with a drawer below the top and above the knees. Nor is a higher table, with which a revolving stool is used, convenient for work with a drawer between the knees and top of the table. The discomfort in the higher tables is no doubt much relieved by the foot-rest supplied. But if the idea is to diminish the height of the table relative to the student's feet by supplying a foot-rest, it would seem as reasonable to reduce the entire height, and make the foot-rest the floor. Place the average student, a beginner in his first year's work, perhaps, on a high stool, with or without a back, above the floor, with a foot-rest or without it, a drawer above the knees, making the sitting position an unnatural one, with a microscope high enough to require a considerable stretch of the back and neck to do work, and two hours will weary any of them but a veteran.

As to length. In Fig. 1 the length is 7 feet, with two drawers on each side. At the best such a table will accommodate four people. With advanced work, and any considerable amount of apparatus, such a table will accommodate two persons, without an increase in width over 3½ feet. Fig. 2 is 8 feet 6 inches long, which will accommodate, with convenience, three on a side. Fig. 3



accommodates two students. Figs. 4, 5, 6, and 8 each accommodate one student. Fig. 7 is 8 feet 6 inches long, accommodates five students, or with crowding, seven.

As to tops. Prof. J. D. Snyder of Leland Stanford suggests that tops be made double, the grain of the boards running in opposite directions. The tables at the University of Iowa are slate-topped. Professor Macbride says, "This makes them heavy and perfectly stable for microscopic work, and contributes greatly to cleanly habits on the part of students—reagents produce no stains whatever. Even if marred they may be easily repolished with a piece of fine sandstone and water."



Fig. 4. *b.* Top of table at University of Illinois, arranged to prevent warping.

Tops of tables in Fig. 7 are made of hard wood about two inches thick, planed and sand-papered, but not varnished. There are no cracks in the top, and whatever is spilled on the table can be easily washed off, or if it remains long enough to stain, the upper surface can be sand-papered and remain as good as before.

Tables in Fig. 8 have the tops of oak,  $1\frac{1}{2}$  inches thick, stained black with logwood and copper sulphate, and coated with paraffin driven into the wood by a hot iron, according to the plan suggested by Prof. Charles Wright Dodge.

As to light. A north light is said by most authors to be preferable to any other. This is certainly not so in the Mississippi valley. A residence of ten years in Illinois, with a south light for work, enables the writer to speak on that

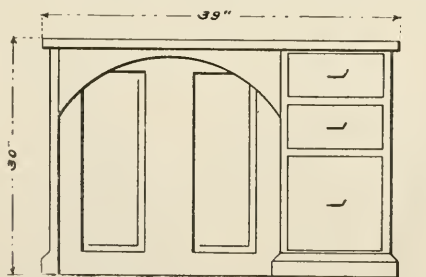


Fig. 5. Side view of table at University of Illinois.

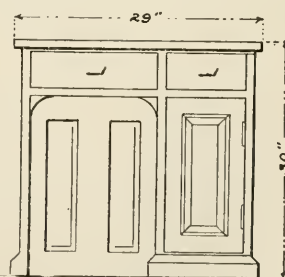


Fig. 6. Side view of table at University of Illinois.

subject. During the spring months the sun will not shine for weeks at a time, and the best light one can get is none too strong. It is better to have too much light a small portion of the time than to have too little a larger portion. My rooms have always had a south and west face, and it has seldom been an inconvenience because of too much light, and many times has permitted work when the opposite facing would have made work impossible.

Long tables may be placed endwise in front of a window, and thus give good light. Fig. 7 gives the best light as far as interference is concerned. Prof. Ward says, "There is very great advantage in the sloping sides, since it enables one to get better light at a distance from the window, and that without the risk of the illumination being interrupted by the movements of the student who sits next to the window."

Short tables, or individual tables, can be bunched, and thus offer the same advantages as long ones. This is true of Figs. 3, 4, 5, 6, and 8. Four tables in a row, in Fig. 8, with the first one next the wall, take 16 feet. Two rows, back to back, accommodate eight students. If placed in front of a window, there is

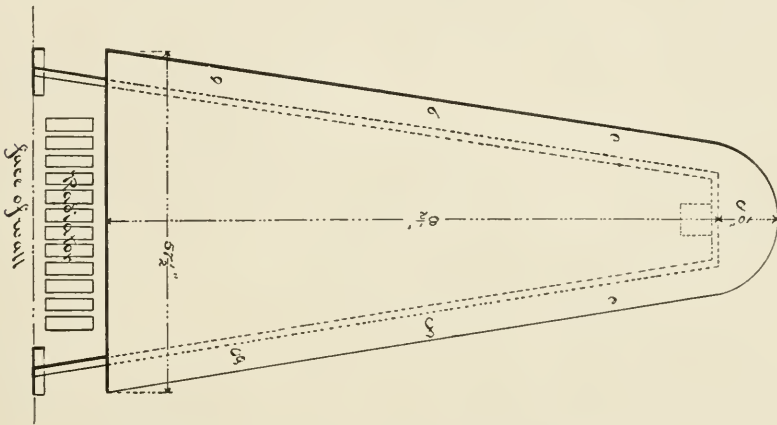


Fig. 7. Top view of table at University of Nebraska.

good opportunity for sufficient light without interference. Short tables have the advantage, that they may be associated in any way to suit the space one has, or to accommodate the needs of the individuals using them. If long tables are placed parallel to windows, some students must sit with backs to the light, or half of the table is unused. With short tables, small space may be utilized where light may be obtained, and at the same time, by bunching, all the conveniences of large tables are secured.

As to drawer space for students. Students in chemistry are provided with plenty of room for flasks, burettes, platinum, beakers, etc., with extra space for large pieces of apparatus. There is no reason why students in biology should not have as much material, apparatus, and space in proportion to the needs in the work as should students in chemistry. It is claimed for the long tables, and by high authority, that it is easier to pass apparatus and books to students when at long tables. Should not the question be, "What is best for the student?" rather than, "What is easiest for the instructor attending?" Moreover, in most of the work in biological lines it should not be necessary to pass books and material. Each student certainly should have his own for his laboratory period, at least, which is usually two hours. The books and material are therefore given at the beginning of the work, and for this it seems it would be no more difficult to hand it to him at one place than at another. This is true whether

the student is engaged in work in general biology, in zoölogy, in botany, in microscopy, in bacteriology, or kindred work. Moreover, until the student becomes sufficiently proficient to launch

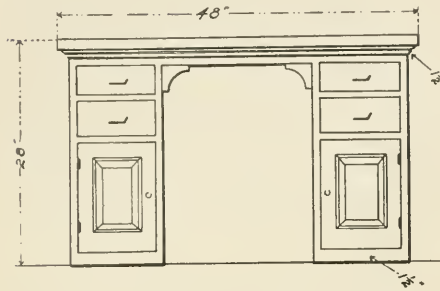


Fig. 8. *a.* Side view of table at University of Montana.

out into work that is more or less investigative, biological work, to produce the best results, is carried on after some definite plan, in some logical order, and with a definite outline for an entire class to follow. This may be in the form of mimeographed notes or by following the plan laid down in some laboratory guide, and in either case the passing of books, material, or specimens should be a very small task.

In following any systematic plan in any line of biological work there is need of material, which should be at the disposal and control of the student in the same manner and in as liberal quantity as in chemistry. There will be necessity for glassware of various kinds, including slips and covers, for nippers, scalpels, scissors, and probes, for reagent bottles, drawing and note paper, pencils, pens, and ink, with occasionally large bottles for storage, possibly tripods, alcohol lamps, or bunsen burners, and a microscope and its accessories. While no one line of work may require all of the articles mentioned, there is no line of work that will not require many of them. If they are not supplied to students the students must supply them themselves. To hand them out from day to day is unnecessary work, and requires almost the same amount of material as when the supply is given at the beginning, and charged to the account of the student.

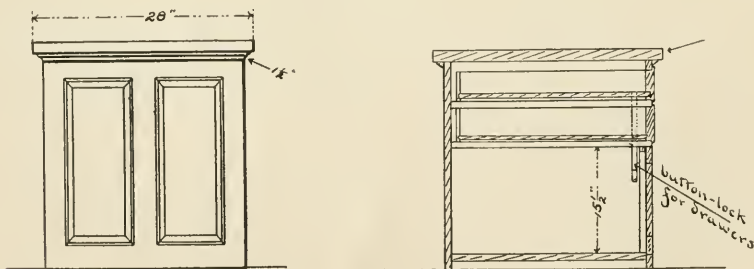


Fig. 8. *b.* End, and sectional view of table at University of Montana.

For this material the student should have room in abundance. It is not good policy to store large and small pieces of glassware together. It is not good policy to keep drawings and notes with glassware and possibly reagents. Obviously, the microscope, a necessity for every student in most biological work, cannot be kept with either of these articles mentioned. Each student should be supplied with at least two spaces for material, and for most lines three spaces

are a necessity, in addition to the storage of the microscope. In Figs. 1, 2, 3, and 7 the drawer space is inadequate, if the tables are used by more than one set of students, working at different times. Of course storage may be supplied elsewhere than in table drawers, as described for Cornell in previous issues of the JOURNAL. But in many places this additional storage place is not to be had, and if it is to be had it is often supplied cheaper in tables than elsewhere. In plan No. 8 the tables may be used by one set of students a portion of the time, by another set at other times, and each student may have at his disposal two drawers and the space below in which to store material. If each student is supplied with a microscope, it may be kept in space with door. Twenty-five tables will thus accommodate fifty students, each student being supplied at the beginning with the working material he must of necessity use. By this means breakage may easily be accounted for, students will exercise care in handling and putting away material, and the work of the instructor attending will be reduced to a minimum. At the close of the term, semester, or year, the material is checked up, turned in, and breakage or loss deducted from the deposit.

In planning tables, these features above-mentioned were taken into consideration. It is not to be denied that individual tastes and varying work may make differences in the effectiveness which one table may offer over another. But it is difficult to see the advantages that long tables have over short ones, and most of the advantages so mentioned may be had by bunching or grouping the short ones. It is worthy of note, however, that the notes accompanying the sketches of tables invariably express satisfaction as a result of the use of the tables. The letter with sketch from which Fig. 1 is made says, "Doubtless improvements could be made, but they serve our purpose very well." With Fig. 3 the letter says, "I like them better than any I have seen elsewhere." With Fig. 4 the letter states that, "Single desks are by far the most convenient." In Fig. 7 the writer of letter says, "I have seen a number of types of laboratory tables, but never one so satisfactory as this." Another gentleman, who has done much work on entomology, as well as other biological lines, both in this country and abroad, says, "In planning tables to be most valuable, have individual desks, low enough to use a microscope vertically, wax top, and plenty of drawer space." It is thus seen that opinions vary. It is hoped that this discussion may be the means of bringing out both the merits and demerits of various forms of tables now in use, so that those planning new equipment may eliminate the unnecessary and undesired, and secure the best to be had for the work to be done on them. So far as I am now able to determine, the only suggestion offered in table Fig. 8, is that the knee space be narrowed a couple of inches or more, making the drawers and space below proportionally wider.

Cost of tables. Fig. 5 is estimated to cost about \$9.00; Fig. 6 about \$12.00; Fig. 8, with panelled door, oak top, as previously mentioned, varnished, waxed, and with two combination locks and four pulls, cost in Montana, each, about \$16.00. Doubtless in Eastern states, where labor is not so expensive, the expense would be from a third to a fourth less. Figures are not at hand to give cost or estimate on the others figured.

MORTON J. ELROD.

University of Montana.



## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

## II.

## THE GENERAL METHOD.

We shall now consider the routine of mounting an object in Canada balsam. While the outline refers more particularly to the paraffine method, the principles are general in their application and must be mastered by every one who desires to make first-class preparations. Several of the topics, like Killing and Fixing, Staining, etc., will be treated in detail when considering the various reagents.

1. *Killing and Fixing*.—Usually the same reagent is used for both killing and fixing. The purpose of a killing agent is to bring the life processes to a sudden termination, while a fixing agent is used to fix the cells and their contents in as nearly the living condition as possible. The fixing consists in so hardening the material that the various elements may retain their natural condition during all the processes which are to follow. This step is one of extreme importance. Take your killing and fixing fluids with you into the field. If you wait until you bring your material to the laboratory you may do some fixing, but it will, in many cases, be too late to do much killing. Always have the material in very small pieces in order that the reagents may act quickly on all parts of the specimens. Pieces larger than one-fourth inch cubes should be avoided whenever possible. For very fine work no part of the specimen should require the reagent to penetrate more than one sixteenth of an inch. In general, the volume of the reagent should be ten to twenty times that of the material. The time required for this process varies with the reagent, the character of the tissue, and the size of the piece. About twenty-four hours is a safe period for chromic acid solutions. While a shorter period might suffice in some cases, even a longer period would probably do no harm.

2. *Washing*.—Nearly all fixing agents, except the alcohols, must be washed out from the material as completely as possible before any further steps are taken, because some reagents leave annoying precipitates which must be removed and others interfere with subsequent processes. Aqueous fixing agents with chromic acid as their principal ingredient are washed out with water; aqueous solutions of corrosive sublimate are also washed out with water, but alcoholic solutions should be washed out with alcohol of about the same strength as the fixing agent; picric acid, or fixing agents with picric acid as an ingredient, must not be washed out with water, but with alcohol, whether the picric acid be in aqueous or alcoholic solution. Running water is best, and where this is not convenient the water should at least be changed quite frequently. The washing-out process usually takes from twelve to twenty-four hours, but it can be shortened about one-half by keeping the fluid lukewarm.

3. *Hardening and Dehydrating*.—After the material has been washed, it is necessary to continue the hardening and also to remove the water. Alcohol is used almost entirely for these purposes. It completes the hardening and

at the same time dehydrates, that is, it replaces the water in the material, an extremely important consideration, for the least trace of moisture, a trace so slight as to be almost imaginary, is nevertheless sufficient to make a preparation poor or indifferent when it might have been excellent.

The process of hardening and dehydrating must be gradual. If the material should be transferred directly from water to absolute alcohol, the hardening and dehydrating would be brought about in a very short time, but the violent osmosis would cause a ruinous contraction of the more delicate parts. Therefore transfer from water to 35 per cent. alcohol, which should act for six to twenty-four hours. Then use 50 per cent. for a similar period. Material may now be placed in 70 per cent. alcohol, where it may remain until ready for use, since 70 per cent. alcohol is a good preservative. Various devices, like constant drips and osmotic apparatus, have been proposed to secure a more gradual transfer. Whether these have any real advantages still remains to be proved. The writer has taken well fixed fern prothallia through the series 35 per cent., 50 per cent., 70 per cent., without the slightest plasmolysis. Such things as fern prothallia, filamentous algæ, etc., can be watched under the microscope as the transfer is made, and if plasmolysis results the series of alcohols may be made closer, *e. g.*, 10 per cent., 20 per cent., 30 per cent., etc. It is said that material left for some time in 70 per cent. alcohol will shrink in spite of good killing and fixing, and it is also claimed that its capacity for staining is diminished. Some recommend that glycerine be added to the alcohol, others prefer to complete the dehydrating process and leave the material in an essential oil, while still others would imbed it and keep it in paraffin. The last is doubtless best of all, but requires such an immense amount of labor that it is impracticable for general purposes. Nearly all of our own material, which is not needed for immediate use, is in 70 per cent. alcohol, unless, of course, the material has been put into formalin or some such reagent which kills, fixes, and preserves all at once.

After the 70 per cent. alcohol, use 85 per cent., 95 per cent., and 100 per cent. successively, allowing six to twenty-four hours for each. The 70 per cent. would probably complete all the hardening which is necessary, but the other three must be used to complete the removal of water.

*Up to this point* the processes are exactly the same, whether the material is to be imbedded in paraffin or celloidin.

4. *Clearing.*—Let us suppose that the material has been thoroughly dehydrated so that not the slightest trace of water remains. If the supposition chances to be contrary to fact, all the work which has preceded, as well as all which is to follow, is only an idle waste of time. The purpose of a clearing agent is to make the tissues transparent, but clearing agents also replace the alcohol. At this stage the latter process is the essential one, the clearing which accompanies it being incidental. The clearing, however, is very convenient, since it shows that the alcohol has been replaced and that the material is ready for the next step.

Various clearing agents are in use. Xylol is the most generally employed, and for most purposes it seems to be the best. Bergamot oil, cedar oil, clove oil, turpentine, and chloroform are all necessary for special purposes.

The transfer from absolute alcohol to the clearing agent should be *gradual*, like the hardening and dehydrating processes. The following is a good method:

- 3 parts 100 per cent. alcohol, and 1 part xylol, 1 to 10 hours.
- 2 parts 100 per cent. alcohol, and 2 parts xylol, 1 to 10 hours.
- 1 part 100 per cent. alcohol, and 3 parts xylol, 1 to 10 hours.

Pure xylol until the material becomes transparent. This may require only a moment, but may require hours. Other clearing agents may be used in the same way instead of the xylol.

5. *The Transfer from Clearing Agent to Paraffin.*—This should also be a *gradual* process. The most convenient method is to place a small block of paraffin in the pure clearing agent with the material. The paraffin dissolves gradually and produces the same result as if a small shaving of paraffin had been added every few minutes for a day or so. During this process the bottle or dish should be kept lukewarm. Six to ten hours, or over night, should be sufficient for this step, although it would seem that material may be kept here for a much longer time without injury. Excellent preparations of the embryo-sac of *Aster* have been made from material which had remained in the xylol and paraffin for nearly three years. No more paraffin should be added than will go into perfect solution. The temperature may be gradually increased so that a much greater amount of paraffin will go into solution, but the paraffin must not be allowed to crystallize.

6. *The Paraffin Bath.*—This step is usually called infiltration, but when the transfer from the clearing fluid to paraffin is made gradually, as has just been indicated, the process of infiltration is already begun. It is now necessary to get rid of the xylol or other clearing agent. This may be done by simply pouring off the mixture of xylol and paraffin and replacing it with pure melted paraffin. The bath should be kept at a temperature about 1 degree C. higher than the melting point of the paraffin. 53 degrees C. is a good temperature for general purposes, but this may be reduced from 1 degree to 3 degrees C. in winter and must often be raised in summer. For special purposes it is sometimes necessary to use a temperature as high as 70 degrees C. If the xylol or other clearing agent is not thoroughly removed the paraffin will be granular or mealy, and will not cut well. It is well to change the paraffin once or twice to make sure that the clearing agent is all removed. Do not waste this paraffin, for the clearing agent can be driven off by prolonged heating and the paraffin is better than ever. Most people use soft paraffin (about 45 degrees C.) for the first half of the time necessary for infiltration, and a harder grade (48 degrees C. to 54 degrees C.) for the latter part of the process. This is a good plan, for soft paraffin melts at a lower temperature, and it is always well to minimize heat.

Some think that it is better not to pour off the mixture of the clearing agent and paraffin, but rather to evaporate the clearing agent by keeping the temperature just high enough to prevent the crystalization of the paraffin. This method has certainly given good results with small, delicate objects.

The time required for infiltration varies with the character of the tissue and the size of the piece. Few things can be well infiltrated in less than an hour.

Lily ovaries require one to four hours; heads of aster at the fertilization period, six to twelve hours. Some claim that even delicate objects, like fern prothallia and the filamentous algæ and fungi, are not injured by a bath of several days if care be taken not to let the temperature rise above 48 degrees C. to 50 degrees C.

7. *Imbedding*.—Material may be imbedded in paper trays, watch crystals, or in apparatus made for the purpose. Embedding L's consisting of two L-shaped pieces (Fig. 6) of brass, type metal, or lead are very convenient. We use a pair of L-shaped pieces with arms three inches long. These furnish a box of almost any required size. A piece of glass serves for a bottom. The tray, Minot, or whatever is used should be slightly smeared with

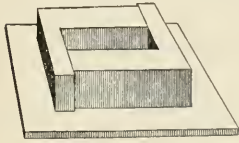


Fig. 6.

glycerine, to prevent sticking. If several objects are to be imbedded in one dish, it is best to have the dish as near the temperature of melted paraffin as possible, otherwise the objects may stick to the bottom and it will be impossible to arrange them properly. Great care should be taken, however, not to have the dish too hot, since too high a temperature not only injures the material, but also prevents a thorough imbedding. Pour the paraffin with the objects into the imbedding dish and cool as rapidly as possible. If paraffin cools slowly it crystallizes and does not cut well.

8. *Cutting*.—Twenty minutes after an object is imbedded it is ready for cutting. Trim the paraffin containing the object into a convenient shape and fasten it upon a block of wood. Blocks of pine three-fourths of an inch long and three-eighths of an inch square are good for general purposes. Put paraffin on the end of the block so as to form a cap about one-eighth of an inch thick. Warm the cap and the bottom of the piece containing the object and press them lightly together; then touch the joint with a hot needle, put the whole thing into cold water for a minute, and it is ready for cutting. Cutting can be learned only by experience, but a few hints may not come amiss: *a.* Keep your knife *sharp*. *b.* Keep the microtome well oiled and *clean*. *c.* Trim the block so that each section shall be a *perfect rectangle*. The knife, which should be placed at a right angle to the block and not obliquely, should strike the *whole edge* of the block at once, and should leave in the same manner. *d.* Sections will usually come off in neater ribbons if the knife strikes the longer edge of the rectangle so that the sections are united by their longer sides rather than by the shorter.

9. *Fixing Sections to the Slide*.—Sections must be firmly fixed to the slide or they will be washed off during the processes involved in staining. Mayer's Albumen Fixative is excellent for this purpose. Formula:

White of Egg, 50cc. (Active principle.)

Glycerine, 50cc. (To keep it from drying up.)

Salicylate of Soda, 1 g. (Antiseptic to keep out bacteria, etc.)

Shake well and filter. It will keep from two to six months. The fixative may be used alone or in connection with the water method. Put a small drop of fixative on the slide, smear it evenly over the surface and then wipe it off with a clean finger or piece of linen until only a scarcely perceptible film remains;



then add several drops of distilled water and float the sections or ribbons on the water. Warm gently until the paraffin becomes smooth and free from wrinkles. Be careful not to melt the paraffin, for the albumen of the fixative coagulates with less heat than is required to melt the paraffin. After the sections become smooth remove the water with blotting paper and allow the slide to dry. The drying will require two or three hours, and a longer period does no harm. If the fixative is used alone, none of this delay is necessary since the sections are merely laid upon the fixative and pressed down gently with the finger.

10. *Removal of the Paraffin.*—To remove the paraffin it is very customary to heat the slide gently until the paraffin melts, and then place the slide in xylol for thirty seconds or a minute. I believe, however, that it is far better to merely warm the slide a little (not warmer than 30 degrees C.) Good xylol will then remove the paraffin in two or three minutes. Even if the slide should not be warmed at all, good xylol should remove the paraffin in five minutes.

11. *Removal of Xylol.*—The xylol may be removed either by absolute alcohol or by the 95 per cent. The absolute alcohol does not seem to be really necessary. For my own work I have two Stender dishes of xylol and two of absolute alcohol. After the xylol has been used for a time I employ it only for removing paraffin, and in the same way use the absolute alcohol only for removing the xylol, while the other two dishes are used only for dehydrating and clearing. In transferring from xylol to absolute alcohol, or *vice versa*, it is well to drain off the superfluous liquid by resting the corner of the slide on a piece of blotting paper. These rather expensive reagents will last much longer if this precaution is taken.

12. *Transfer to the Stain.*—Stains are aqueous or alcoholic, and alcoholic stains are of various strengths. If an aqueous stain be used, the slide should be passed successively through the alcohols 95 per cent., 85 per cent., 70 per cent., 50 per cent., and 35 per cent., allowing each to act for about thirty seconds, after which the slide is put into the stain. (In many cases it is sufficient to put a few drops of the stain on the slide with a pipette.) From the stain the slide is passed back through the various grades of alcohol, allowing it to remain about thirty seconds in each as before. If the stain be alcoholic of about 70 per cent. strength, the process is somewhat shorter, for the slide goes into the stain from 70 per cent. alcohol, and goes back into 70 per cent. alcohol from the stain. The rule is to transfer to the stain from the alcohol which is nearest the strength of the stain. Exceptions to the rule will be considered when treating of the various stains.

13. *Clearing.*—After the sections have been stained, passed back through the various grades of alcohol, and have been thoroughly dehydrated in absolute alcohol, they are cleared or made transparent by means of xylol or some other clearing agent. The clearing agent must be a solvent of balsam. From thirty seconds to five minutes will be sufficient for clearing any kind of sections.

14. *Mounting in Balsam.*—After the sections are cleared, wipe the slide on the side which does not bear the sections. Put on a drop of Canada balsam and add a clean, thin cover. Before you put the cover on, pass it through the flame of an alcohol lamp to remove moisture, for it would be a pity indeed to injure a preparation at this stage of the process. Add a label and the mount is complete.

(To be Continued.)

## Carnoy and Lebrun's Observations on Fertilization in *Ascaris Megalocephala*.

The true interpretation of the process of fertilization is fundamentally necessary to the solution of many problems of heredity. Observation has not yet settled conclusively the ultimate fate of the constituents of the spermatozoid or the part they play in this process. The least light on this subject ought therefore to be of far-reaching interest.

*Ascaris megalocephala*, on account of its large spermatozoa and few chromosomes, is a most favorable object for the study of fertilization, and indeed has become classical: but widely different results have been reached, varying with the methods employed. The technical difficulties involved hinge about fixation and embedding. Flemming's fluid, Hermann's, nitric acid, sulphurous alcohol, and many other killing fluids of unquestionable value elsewhere fail at the dense egg membrane of the nematode. Carnoy and Lebrun, in their paper which appeared in "La Cellule" nearly two years ago, report the excellencies of Gilson's fluid, which is composed of equal parts of glacial acetic, chloroform, and absolute alcohol with corrosive sublimate to saturation. The glacial acetic has a softening effect on the egg covering; the chloroform, being very rapidly penetrating, is said to anesthetize the protoplasm; while the other two constituents fix very promptly and very faithfully the different cell structures. In their use of this fluid unusual precaution was taken to insure its full effect by slitting the uterine tubes in many places. Having acted for some ten minutes, the fluid was followed by weak alcohols, until all traces of acetic and chloroform odors had disappeared, then by increasing alcohols to eighty per cent. where the material was preserved. Embedding in paraffin of a melting point below 50° C., after immersion for one to two minutes at most, was the only admitted alternative with the much preferred celloidin method. Three changes (of increasing thickness) of the latter, each used for fifteen days, were found necessary. Staining was by Heidenhain's iron hæmatoxylin method.

Taking up first the phenomena of maturation, they attack the problem of the nature of the polar figures—whether they are true *kineses*, as that term is generally understood. The view most widely accepted may be summarized after Sobotta as follows: (1) no centrosomes in polar figures; (2) no true asters; (3) spindle truncated—fibers not meeting at the centrosome. Boveri and Sobotta hold that there could be no centrosome in the maturing ovum because it has already been lost from the cytoplasm of the ovocyte, and this essential element will be lacking until it is supplied by the entering spermatozoid. Lebrun's discovery of "polar corpuscles" or centrosomes in *Ascaris* (1892) has been confirmed by Haecker, Sala, v. Erlanger, and others. The authors claim for Gilson's fluid the preservation of aster rays, though not so distinct as in ordinary kineses. They thus supply another element of a true kinesis; and they oppose the deduction of Sobotta, that because the spindle is not pointed it is therefore not a true one.

In the spermatozoid are distinguished a true reticulum, "easily seen in the 'head' and along the periphery of the 'cone,'" and a central refractive body composed of a myosic enchylema which dissolves gradually after entrance into the ovular cytoplasm, thereby unmasking the reticulum in this part of the cone. Boveri, v. Beneden, Kultschitzky, and v. Erlanger have maintained that the whole spermatozoid disappears by this "digesting" action, some of them adding that it furnishes only the needed centrosome. Kostanecki, Siedlecki, and the authors agree that the sperm is neither thus lost nor cast out, but adds itself to the ovum—"impresses its seal" on the egg. As the enchylema disappears the spermatric reticulum fuses with the ovular reticulum in a *plage du fusion* which is filled with enchylematous granules (=Boveri's archoplasm). The trabeculae of the *plage du fusion* are seen to be continuous with the cytoplasmic trabeculae, and since there is no residual figure—no remnant of the spermatozoid, it must have fused with the egg, the result being an element of mixed nature.

Segmentation is accomplished under the agency of "corpuscles," which answer in all but origin to the centrosomes of other authors. There are formed, at the expense of the chromatin of the pronuclei, two to four small bodies figured by others as achromatic nucleoli. These reduce by fusion to one in each pronucleus. They then escape through the nuclear membrane before its dissolution, and take position at opposite ends of the forming segmentation nucleus, where they function as centrosomes. By chemical influence on the surrounding reticulum, the trabeculae are thrown into radiating fibers, which thus compose the asters and the spindles. The attraction spheres of v. Beneden, thought by him to be permanent, are these aster formations filled with granules. The centrosome, having accomplished its purpose, disappears by absorption and new ones are formed by the daughter nuclei for the next segmentation.

In the variety *univalens*, an important point is noticed in the re-formation of the nuclei. Each nucleus gets a male and a female V, which come in the course of regeneration to be crossed after the manner of the wickets of a croquet "basket." By disintegration of the middle parts these break in two, and then fuse conjugally (spermatric with ovular portions) to form the nucleinic cord. The disintegrated middle portions form the nucleoli which become the centrosomes of the succeeding cleavage. There is a possibility here of maternal and paternal centrosomes. Subsequently, when chromosomes are formed from the loose skein stage, the division is such that each of the two is again of mixed nature.

According to the most generally accepted theory of fertilization: (1) the egg and sperm are each something short of a complete cell—the egg lacks a centrosome, the sperm, cytoplasm; (2) each complements the other, the deficiencies being made good by fusion; (3) the centrosome is a permanent organ of division introduced by the sperm, and perpetuated from one generation of cells to another by division. The first proposition is refuted by Carnoy and Lebrun, by pointing out that every cell loses its centrosome after division—a centrosome not being found in somatic cells at rest. The ovocyte is therefore not peculiar in this respect. Second, the deficiency could not be filled by the male element for the reason that it has no centrosome until its

nucleus forms one. What observers have mistaken for a centrosome is either a nucleolus just emerged from the nucleus or a remnant of the spermatic enchylema. The last proposition is answered by the authors' assertion that the centrosome disappears after each karyokinetic act. The true conditions of fertilization are, therefore: (1) That the female element lacks, not a centrosome, since it can supply one from its own nucleus, but a certain amount of chromatin. In maturation it has lost three-fourths by weight, if not always in number, of this material. The male element also, as a result of the last two divisions in spermatogenesis, contains only one-fourth of the normal amount in the sperm mother cell. Now, if we suppose that during the repose previous to segmentation, there is a doubling up in the amount of chromatin as occurs in other nuclei in repose, the necessary quantity will be at hand before the first cleavage. (2) The presence of the spermatozoid is necessary that the two centrosomes (maternal and paternal?) may be formed. A third condition seems, from evidence furnished by experimentation, to be necessary, namely, that before the female cytoplasm has the power to divide, it must be acted on by the male cytoplasm. The reason the female cell does not proceed to form the new individual before fertilization is therefore that it lacks: (*a*) half the normal amount of chromatin, (*b*) a spermatic centrosome, (*c*) a chemical working-over of its cytoplasm. Fertilization is, then, a *fusion* of the two cells, nucleus with nucleus, and cytoplasm with cytoplasm, for the purpose of supplying what is wanting in the female cell.

The bearing of such phenomena on the theory of heredity would be evident. Since heredity may be defined as the faculty which fertilized eggs have of producing beings similar to both parents, and since, as they believe, the two sexual elements completely fuse, one with the other, Carnoy and Lebrun conclude that maternal and paternal characters are transmitted with equal "force" *because* the fertilized egg is a mixed element, and that the "substratum" of heredity is not the chromatin alone nor the nucleus alone, but the whole cell.

J. R. MURLIN.

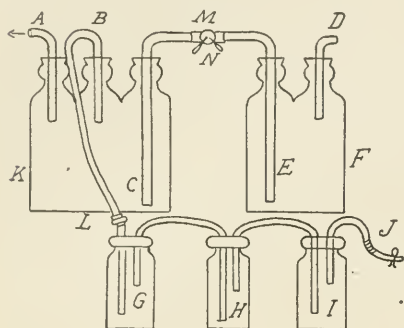
University of Pennsylvania.

## A Simple Hydrogen Generator.

Some months since, while at the Pasteur Institute in Paris, I noticed the apparatus devised, I think, by Professor Roux, which they use for the evolution of hydrogen. This seemed so much more simple, inexpensive, and permanent in its working than Kipp's, that I thought it worth calling attention to in these columns: *k* is a large three-mouthed Wolff bottle more than half full of chemically pure zinc; *f* is a two-mouthed Wolff bottle filled with sulphuric acid and water. To operate the apparatus a rubber tube connected with a water vacuum pump is attached to glass tube *a*. The vacuum thus created in bottle *k* causes the acid mixture in bottle *f* to pass through tubes *e* and *c* into bottle *k*. Hydrogen is at once evolved, which passes through tube *b*, with rubber connection, to bottle *g* containing a solution of caustic potash, to bottle *h*, with a solution of



silver nitrate, and to bottle *i*, holding merely water. They do not employ pyrogallie acid for purification. Evolution of the gas can be controlled by a pinch-cock, either at *l* or at *j*. As soon as the desired amount of hydrogen has passed, closure of the pinch-cock, or stop-cock, at *l* or *j*, causes the hydrogen in *k* to be developed under pressure. This pressure soon forces back through the tubes *c* and *e* all the sulphuric acid and water, and the zinc is thus left dry until the apparatus is again wanted. Tube *d* is simply for the inlet of air when the acid mixture is pumped out, and the exit of the same air when the original contents come back.



This apparatus is so much cheaper than Kipp's, and is so much easier kept in permanent operation, that it at once recommends itself.

Again, this apparatus can easily be made by any one, and that cannot be said of Kipp's. For instance, the Wolff bottles are not necessary. Two wide-mouthed bottles would answer, through the cork of one of which three glass tubes could be passed, and of the other two. Neither is the water pump necessary. By blowing into a rubber tube connected with *d*, or, better still, attaching a bicycle pump to it, one could force the contents of bottle *f* into bottle *k*. *m* is a small piece of rubber tubing seized by a pinch-cock, *n*, and as this is the only piece of rubber in the whole apparatus exposed to the acid mixture, but little in the way of repairs is ever needed.

This form of apparatus, of course, will do just as well for the evolution of sulphuretted hydrogen as for the purpose just described.

ERNEST B. SANGREE, M. D.

Vanderbilt University, Nashville, Tennessee.

## BOOK NOTICES.

**Morell, Harry, M. D. C. M., F. R. M. S.** Trinity University, Toronto, Ont. Records of Urinary Examinations. J. S. Burr & Co., Hartford, Conn.

This is a laboratory note-book for systematically recording the results of urinary analysis. Provision is made for one hundred records in duplicate,

one sheet being detachable, the other remaining in the book. The record provides for notation as to physical characters and for physiological and pathological constituents in solution, and also the results of microscopical examination, with a special paragraph for bacteriological record. The results of centrifugal analysis may also be recorded in connection with other tests. The author says: "One examination of the urine is rarely a criterion in making a diagnosis of any disease. The physician can at once see by looking back whether the albumen or sugar in a certain case is increasing or diminishing; or by comparison of examinations he can make a more accurate determination of the

conditions present. The hospital pathologist, after completing an analysis and noting it, separates the sheet at the perforation, sends it to the ward, and retains the carbon copy in the book, where it may be referred to at any time." The volume is serviceably bound in oilcloth, which is not injured by accidental contact with fluids, and it may be washed if soiled. It contains in the back an alphabetical index for patients' names.

L. B. E.

**Farrington & Wall.** Testing Milk and its Products. Mendota Book Co., Madison, Wis., 1898.

This is a well arranged manual for practical use in testing milk. It gives full descriptions of the different meth-

ods of making tests, with the apparatus used, and in addition there is a chapter on the composition of milk and its products, and a chapter with directions for making chemical analyses. There are also methods and tables for calculating dividends, and many suggestions of practical use to the dairyman.

E. M. B.

**Novy, Frederick G., M. D.** University of Michigan. Laboratory Work in Bacteriology. Second Edition. George Wahr, Ann Arbor, Mich., 1899.

The second edition of Dr. Novy's "Laboratory Work" is greatly enlarged and provided with many new illustrations. The work is intended as a text-

book of laboratory work for medical students, and conforms closely to the actual work in Dr. Novy's classes in the Hygienic Laboratory of the University of Michigan, which, in itself, is enough to make it of interest to every bacteriologist.

L. B. E.

**PERMANENT MOUNTS OF POLLEN.**—When only external characters are desired, very beautiful permanent mounts can be made in the following manner: Put a drop of albumen fixative on the slide and spread it out in a thin layer. Sprinkle the fresh pollen on this and then put the slide into a stender dish of absolute alcohol to which equal parts of a small amount of anilin safranin and gentian violet have been added. About 0.1 gram of each to 100 cc. of the alcohol is the proper amount. After five or ten minutes transfer to xylol, and when cleared mount in Canada balsam.

JOHN H. SCHAFFNER.

Botanical Laboratory, Ohio State University.

**CONCERNING FRESH WATER AQUARIA.**—Mr. Piper's suggestion in the January number of the JOURNAL, while effective for the purpose he claims, seems to possess no advantages over a plan I have adopted for some years, viz., to cover the surface of the water with *Salvinia natans*. This floating plant effectually protects the surface from the accumulation of bacterial zooglæa, although the *Salvinia* itself is continually attacked by moulds; it is so energetic, however, in its growth that its decay is not proportionate to its increase. In one of my aquaria containing vigorous plants of *Cabomba rosæ-folia* and *Carolineana*, and whose surface is covered with the *Salvinia*, the algæ *Spirogyra* and *Cladophora* are exceptionally prolific; in another containing a great mass of the aquatic moss, *Amblystegium riparium*, and with an uncovered surface, I am unable to grow any algæ. Doubtless, plants such as *Azolla* or *Lemna* would perform the same functions, but these both have their periods of decay when they disappear altogether, while the *Salvinia* is in evidence the year around.

Fort Hamilton, N. Y.

A. H. GARDNER.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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\* \* \*

THE gradual decline of popular interest in the microscope is in striking contrast with the increase in its use by technicians in many branches of science and industry. It has been variously attributed to the phenomenal increase in the use of the camera and bicycle, but while these may have affected the younger element to a certain extent, the man of middle age and older is not, as a rule, a camerist or cyclist, and is not likely to be diverted from so interesting and withal profitable and congenial a pursuit as microscopy by anything so totally different.

The cause must lie deeper, and our observation leads to the belief that a lack of sympathy between the advanced men of science and their humble followers has been largely instrumental in producing the present indifference.

The increase in the number, convenience, and mechanical excellence of microscopical appliances, and the constant decrease in their cost, should have had the same stimulating effect upon microscopical work as has the production of suitable apparatus on photography.

Is it not the lack of suitable teachers? Teachers whose attainments in science are beyond question, investigators whose researches place them in the front rank, and yet whose interest in the education of the public to an appreciation of the basic principles of the sciences whose exponents they are, and of the value of scientific observation as a means of broadening and bettering the mental status, is equal to their interest in the solution of intricate biological problems.

There is an abundance of treatises by capable writers, but they take up the subjects where books for the beginner should end.

Books which begin at the beginning and which deal with facts in a perfectly scientific, accurate manner, and yet with the utmost simplicity, which will aid the beginner without confusing, which will develop his powers of observation without detracting from the thing itself, and which will tell him the what, where, and how of microscopical work and the microscopical world, will be appreciated by the thinking laity. The labors of the specialist are fruitless if they do not contribute something which is useful to the great masses outside his chosen circle.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Popta, Canna M. L.** Beitrag zur Kenntniss der Hemiasci. Flora, 86: 1-46. pl. 1 and 2, 1899.

The fungi belonging to the Hemiasci have been variously classified. De Bary (1865) placed *Protomyces* with the Ascomycetes as their simplest form, but later concluded that it belonged to the Ustilaginæ. Schröter united *Protomyces* with *Endogone* into an independent order, Protomycetes, following the Zygomycetes and Oomycetes. In Rabenhorst's Kryptogamen Flora, *Thelobolus*, now usually classed with the Hemiasci, is placed under the Pezizaceæ. Brefeld includes in the Hemiasci three genera, *Ascoidea*, *Protomyces*, and *Thelobolus*, and places the group between the Phycomycetes and the Ascomycetes. This position is given because the sporangia agree more nearly with those of Phycomycetes than with the asci of Ascomycetes, while the vegetative characters show more resemblance to those of Ascomycetes. The present work was undertaken in the hope that a study of the development of the sporangia and spore formation might shed some light upon the subject. *Ascoidea rubescens* Brefeld and Lindau, *Protomyces bellidis* Krieger and *Protomyces macrosporus* Unger were studied in detail. A chapter is devoted to a comparison of the sporangium development of the Hemiasci with that of the Ascomycetes and Phycomycetes. The conclusion is that in their spore development the Hemiasci do not constitute a single group, but rather that a part (*Ascoidea*) shows more analogy to the Ascomycetes, while others (*Protomyces*) are nearer the Phycomycetes.

C. J. C.

**Guignard, L.** Sur la formation du pollen et la réduction chromatique dans le *Naïas* major. Comptes rendus des séances de l'Acad. des Sci. 128: 1-5, 1899.

The number of chromosomes in *Naïas major* is twelve in the sporophyte and six in the gametophyte, the lowest number yet reported in any flowering plant. This paper deals with the two mitotic divisions by which the four pollen grains are formed from the pollen mother cell. In the prophase of the first division the spirem splits longitudinally and segments into six primary chromosomes, each of which consists of two pieces. Each of these pieces during the succeeding contraction and growth shows a double row of granules, a preparation for a second splitting, so that the primary chromosome is to be considered quadruple. As the primary chromosome separates into its two parts (secondary chromosomes), the splitting already presaged by the division of the granules begins to take place, but is not entirely completed since the two pieces remain united at their extremities, thus forming a V with its apex in contact with the spindle threads. Each of the daughter nuclei receives six double (secondary) chromosomes. In the second division six V-shaped chromosomes appear. At the point of the V there is an interruption in the linin support, and everything



favors the conclusion that these are the secondary chromosomes of the previous division which have not lost their individuality. No longitudinal division takes place at this time, but the second division seems to consist essentially in the separation of the two parts of the V-shaped secondary chromosome. Thus the two divisions merely distribute the four parts of the quadruple chromosome (tetrad) which were already defined in the prophase of the first division. It is evident that there is no qualitative reduction.

This description agrees with Farmer's account of *Pallavicinia*, Brauer's of *Ascaris*, and Meves' of *Salamandra*. Guignard believes that the facts observed agree with Belajeff's account of *Iris*, although that writer has given a different explanation of the origin of chromosomes in the pollen mother cell. It seems strange that no mention is made of the work of Miss Sargent, who, two years ago, both figured and described a second fission of the chromatin granules in the pollen mother cells of *Lilium Martagon*, and called attention to the quadruple nature of the primary chromosome.

C. J. C.

**Mottier, D. M.** Ueber die Chromosomenzahl bei der Entwicklung der Pollenkörner von *Allium*. Ber. d. deutsch. bot. Gesell. **15**: 474-475, 1897.

In *Allium fistulosum* Ishikawa found the reduction in the number of chromosomes taking place in the sporogenous cells some time before the formation of pollen mother cells. The karyokinetic figure concerned in the reduction was heterotypic. Ishikawa also found a transverse splitting of the chromosomes at the second division of the pollen mother cells. Dr. Mottier finds that the reduction in the number of chromosomes takes place in the pollen mother cell, that the karyokinetic figure concerned is heterotype, and that in the second division there is a longitudinal splitting of the chromosomes. If Ishikawa's results are correct they are certainly very exceptional.

C. J. C.

**Berthold, G.** Die geschlechtliche Fortpflanzung der eigentlichen Phaeosporien. Mittheil. d. Zool. Stat. Neapel. **2**: 401-413, pl. 17, 1881.

**Oltmanns, F.** Ueber Scheincopulationen bei Ectocarpeen und anderen Algen. Flora. **83**: 398-414, pl. 7, 1897.

**Berthold, G.** Bemerkungen zu der vorstehenden Abhandlung von Fr. Oltmanns' Ueber Scheincopulationen bei Ectocarpeen und anderen Algen. Flora. **83**: 415-425, 1897.

**Oltmanns, F.** Ueber Sexualität der Ectocarpeen. Flora. **86**: 86-99, 1899.

In the earlier work by Berthold upon the Ectocarpaceæ, made familiar to us by the reproduction of his figures in the text-books, a female gamete of *Ectocarpus siliculosus* is represented as attached to several male gametes, with which it is said to fuse. This process is often cited as illustrating a very primitive condition in the evolution of sexuality. Two years ago Oltmanns called the accuracy of Berthold's obser-

vation in question. According to his own work upon *E. criniger*, Oltmanns says Berthold saw, not fusing gametes, but infusoria, which captured and ate the Ectocarpus swarm-spores. Berthold immediately replied, defending his former position, and insisted that his preparations, which he had loaned to Oltmanns, were not susceptible of any interpretation other than the one he had already given. Recent work by Oltmanns has led him to abandon his former belief and to announce his complete concurrence with the views of Berthold. This later work is upon *Ectocarpus siliculosus*, which was studied also by Berthold. He

variation in question. According to his own work upon *E. criniger*, Oltmanns says Berthold saw, not fusing gametes, but infusoria, which captured and ate the Ectocarpus swarm-spores. Berthold immediately replied, defending his former position, and insisted that his preparations, which he had loaned to Oltmanns, were not susceptible of any interpretation other than the one he had already given. Recent work by Oltmanns has led him to abandon his former belief and to announce his complete concurrence with the views of Berthold. This later work is upon *Ectocarpus siliculosus*, which was studied also by Berthold. He

indeed finds infusoria, as before, but he also finds gametes fusing, and describes the process quite fully. Several male gametes become attached to the female, one of them finally fusing with it. In material collected in the morning this generally occurs before noon. Nuclear fusion is usually completed before night. The fusion of the female with more than one male gamete is very rare, and the author gives us no information as to whether, in such a case, the second male nucleus unites with the others or whether it merely disintegrates. The second chromatophore in the normal zygote is said to persist. We may then say that the Ectocarpaceæ show sexual conditions in all stages of transition, from isogamy in the lower species, through such forms as *Ectocarpus siliculosus*, where there is hardly more than a physiological differentiation of the gametes, to the distinct heterogamy of *E. secundus*, where the two gametes differ considerably in size. The "neutral" swarm-spores, almost always in the plurilocular sporangia with the gametes, arose secondarily, by the failure of the sexfusion.

W. D. MERRELL, Chicago.

**Magnus, P.** Der Mehlthau auf *Syringa vulgaris* in Nordamerika. Ber. d. deutsch. bot. Gesell. 16: 63; pl. 2, 1898.

The lilac mildew *Microsphaera alni* is one of the most familiar fungi in the United States. This paper calls attention to the fact that in Germany, where the lilac has been cultivated for a much longer time, no species of *Microsphaera* is ever found upon it.

C. J. C.

**Pammel, L. H.** The Histology of the Caryopsis and Endosperm of Some Grasses. Trans. Acad. Sci. of St. Louis. 8: 199-220, pl. 17-19, 1898.

A detailed description is given of the anatomical characters of various species and, at the close, a synopsis of anatomical characters for the tribes. In the latter the features are starch grains, aleurone cells, testa, and pericarp. The different tribes present wide structural differences. Compound starch grains seem to be quite general in grasses, the endosperm always contains protein except in the aleurone layer, and fat is present in small amounts. The figures, which were drawn by Miss King, illustrate very clearly the points described in the text.

C. J. C.

**Farlow, W. G.** Some Edible and Poisonous Fungi. U. S. Dept. of Agriculture. Div. Veg. Phys. and Path. Bull. No. 15, pp. 453-470, pl. 21-30, 1898.

This excellent article appeared in the Yearbook of the Department of Agriculture for 1897, but the demand for literature on this subject has been so great that it was found desirable to republish the paper in the form of a bulletin. After describing the mode of growth of toadstools (including mushrooms) and the characteristic modifications of fungi, rules are given for the determination of the edible gill-bearing fungi. Poisonous species are also carefully described. Other topics are: less common edible fungi; tube-bearing fungi; teeth-bearing fungi; some other edible forms; morels and truffles; puffballs. In the summary, the following rules are given for the benefit of the beginner:

"(1) Avoid fungi when in the button or unexpanded stage; also those in which the flesh has begun to decay, even if only slightly. (2) Avoid all fungi which have stalks with a swollen base surrounded by a sac-like or scaly envelope,

especially if the gills are white. (3) Avoid fungi having a milky juice, unless the milk is reddish. (4) Avoid fungi in which the cap, or pileus, is thin in proportion to the gills, and in which the gills are nearly all of equal length, especially if the pileus is bright colored. (5) Avoid all tube-bearing fungi in which the flesh changes color when cut or broken, or where the mouths of the tubes are reddish, and in case of other tube-bearing fungi experiment with caution. (6) Fungi which have a sort of spider web or flocculent ring around the upper part of the stalk should in general be avoided."

The clear descriptions and excellent plates make this a paper of great practical value to every lover of mushrooms.

C. J. C.

#### RECENT LITERATURE.

- Anderson, A. P.** The Asparagus Rust in South Carolina. S. C. Agric. Exp. Station Bulletin 38, Feb. 1899.
- Bruchmann, H.** Ueber die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien, und zwar über die von *Lycopodium clavatum*, *L. annotinum*, *L. complanatum* und *L. Selago*. 7 pl., Gotha. Perthes, 1898.
- Balicka-Iwanowska G.** Contribution à l'étude du sac Embryonnaire chez certain Gamopétales. *Flora*. **86**: 47-71, pl. 3-10, 1899.
- Dangeard, P. A.** Mémoire sur les Chlamydomonadées en l'histoire d'une cellule. *Le Botaniste*, 6th série, pp. 65-292, 1899.
- Fink, Bruce.** Contribution to the Life History of *Rumex*. Minnesota Bot. Studies, Second Ser. Pt. II, pp. 137-153, pl. 9-12, 1899.
- Ramaley, Francis.** Comparative Anatomy of Hypocotyl and Epicotyl in Woody Plants. Minnesota Bot. Studies, Second Ser., Pt. II, pp. 87-136, pl. 5-8, 1899.
- Tilden, J. E.** The Study of Algae in High Schools. *Plant World* **2**: 59-63, 1899.
- Wieland, G. R.** A study of some American Fossil Cycads. Part I. The male flower of Cycadeoidea. *Amer. Jour. of Science*, **7**: 219-226, pl. 2-4, 1899.
- Zacharias, O.** Das Plankton des Arendsees. *Biol. Centralb.*, **19**: 95-102, 1899.

### ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
Agnes M. Claypole, Sage College,  
Ithaca, N. Y.

#### CURRENT LITERATURE.

**Peter, Karl.** Das Centrum für die Flimmer und Geisselbewegung. *Anat. Anz.* **15**: No. 14; pp. 271-283; 4 figs. in text.

The author refers to recent work done on the question concerning the dynamic center of ciliary motion. He carries on his studies to determine whether it is situated in the nucleus, protoplasm or in some part of the cilium itself. In view of the tendency of modern work to look on the centrosome as the dynamic center, previous workers have suggested the possible homology of the basal bodies on the cilia with that part of the cell, on purely theoretical grounds.

The author took the intestinal epithelium of *Anodonta* for his studies, and after much experimenting found the following the best method of treatment: cutting through the foot muscles of the animal when immersed in normal salt solution freed in part the intestine. A piece of this was then put on a slide and cut into fragments with a sharp knife; the cutting being done in all possible planes. In spite of the apparent roughness of the process it gave very good results, cells being quickly and thoroughly isolated and cut in many ways.

The first point considered was the relation of the nucleus to ciliary motion ; in every isolation preparation many denucleated pieces were present, which showed most complete and natural ciliary motion. From this and other evidence the author concludes that ciliary motion is entirely independent of the nucleus ; in this opinion he is supported by Verworn's studies on the Protozoa. The second question, of the dependence of the ciliary motion on the protoplasm, was determined by studies on denucleated pieces of the cells. Pieces showing vigorous ciliary motion undergo changes ; the protoplasm still remaining shrinks together to form a more spherical mass, and in so doing draws away from the free ends of the cilia, leaving more exposed the so-called ciliary "roots," their continuations into the cell substance. During this process no change occurs in the nature of the ciliary movement ; it continues as before. Even stronger evidence comes from finding these ciliary "organs" freely isolated, showing no trace of protoplasm around them, but still possessing movement in some of the cilia.

Having reduced the seat of action to the ciliary "organ" itself, the author brings evidence to prove that the center of force lies not in the cilia themselves, as broken from the roots, they do not move. If the roots are broken away the cilia still move as usual, so that the dynamic center is finally placed in the "basal body" of the cilia, which lies just within the cell limits in a normal condition. His carefully worked out study gives strong evidence for the idea of the basal bodies being the dynamic centers for ciliary motion ; moreover these bodies are most likely truly related to the centrosome in structure.

A. M. C.

**Erlich, P., u. Lazarus, A.** Die Anämie Abth. normale und pathologische Histologie des Blutes ; ueber die Darstellung und Bedeutung der Zellgranula. Spec. Pathol. und Therapie von Nohuagel. 8 : p. 1-13. 1898. Abst. in Zeit. f. wiss. Mikros. 15 : 3.

The authors are considering the best method of fixing and differentiating the many kinds of granules found in normal blood corpuscles. The method of drying on the cover-glass and staining

with the tri-acid stain gives uniformly good results. Erlich had earlier concluded that different blood cells contained granules of different chemical natures, not by stains, but by their reaction to fixers. The value of the drying method lies in the unaltered chemical condition of the cells, heat acting as an instantaneous fixer. Another quick method to preserve the granules is by "vital staining" : stains acting during the life of the cell. Erlich first used methylen blue for this purpose, and later neutral red. This last stain possesses a great affinity for many of the granules, and can be used on the higher animals by subcutaneous or intravenous injection. In tadpoles or other aquatic animals, it is enough to put the animals in a very weak solution of this stain (1 : 50,000 or 1 : 100,000), or leaving small pieces of tissue in normal salt with a trace of neutral red until the object is slightly reddened. Beautiful results are obtained, in which the protoplasm and nucleus are uncolored, only the granules being affected. Artefacts are not entirely excluded by this method, but the experienced worker can readily detect them. By combining neutral red and methylen blue, Erlich found that almost all the granules took the red stain, those of the plain muscle from the alimentary tract alone showing intensely blue color. A third stain gave further



powers of distinguishing the kinds of granules found in living cells. The authors consider these granules as specific cell secretions which can be discharged: the "mast cells" showing this feature most distinctively.

A. M. C.

**Held, H.** Structure of Nerve Cells and Their Processes. Arch. f. Anat. u. Physiol. Anat. Abth. pp. 204-294; and Supplementband, pp. 273-305; 1897;

The author in these two papers presents the results of a study of the finer structure of nerve-cells by the application of various fixing reagents. As a result of his investigations, he concludes that the corpuscles of Nissl in nerve-cells are not present in normal living cells, but first appear upon treatment with acids, either formed by post-mortem changes or present in the fixing fluid. As to the structure of the mass of the cell-body of nerve-cells, the author commits himself to the alveolar theory of Bütschli. In the axis-cylinder the same structure is present, the meshes prolonged in one direction, while the transversé walls are very delicate and short, giving to the axis-cylinder the appearance of a longitudinal fibrillation, so often described as constituting the real structure. The alveolar structure of the cell-body and the axis-cylinder process (neurite) was demonstrable by most of the methods, but unless a small cone of light and a less refractive mounting medium—such as water or glycerine—were employed, the transverse connections were not apparent, and the appearance was of a true fibrillar structure. Of the fixing fluids tried, Van Gehuchten's alcohol-chloroform-formic acid mixture were found to be most useful in the study of the structure of the axis-cylinder. A double stain with erythrosin and methylen blue was largely employed; likewise, Heidenhain's iron hæmatoxylin.

B. F. KINGSBURY.

**Kofoed, C. A.** Some Important Sources of Error in the Plankton Method. Science, N. S. 6: No. 153.

In the Hensen method, a net of silk bolting cloth is drawn vertically through the water, and the catch is multiplied by some factor, the coefficient of the catch, to determine the amount of plankton in a given column of water. The coefficient, calculated by Hensen for a series of velocities, is applied without regard to the character of the plankton. Experiments show that the coefficient varies with the amount and nature of the catch, the straining power of the net being affected by clogging in the case of heavy planktons. The effect of progressive clogging is such that from 84 per cent. to 96 per cent. of the catch is calculated to be taken in the first fifteen meters of a thirty-meter haul. Hensen and others state that with the finest bolting cloth only a few organisms can pass through the meshes, but Kofoed finds that the struggles of the organisms and the pressure of the water aid the escape of the planktons, and that in the pumping method of collection the net retains from 5 per cent. to less than 1 per cent. of the total number of forms present, exclusive of bacteria.

E. M. B.

**Biedl, A.** Ueber das histologische Verhalten der peripheren Nerven und ihrer Centren nach der Durchscheidung. Wiener. klin. Wochenschr. 10: No. 17, pp. 389-392. Abstract in Zeit. f. wiss. Mikros. 15: 3; 1897.

The author studied the peripheral and central degeneration of nerves on rabbits and dogs by resecting 1 to 1.5 cm. pieces of the nerves. The dogs were killed quickly in chloroform, five, ten, and eighteen days afterwards, and the

rabbits three, eight, and twenty-eight days. The most favorable time to determine nerve centers by this method was in from fourteen to eighteen days after the section of the nerve. The tissues were hardened in part, in alcohol, to be used after in Nissl-stain, and part in Marchi's fluid. In one case the spinal cord was put in a 2.5 per cent. solution of formalin, and single pieces either in alcohol according to Nissl, or left for a short time in Müller's fluid, and stained according to Marchi. To stain the ganglion cells, Nissl's methylen-blue method was used, or the same author's magenta red and Senhossik's thionin. The last method gave very good results, but methylen blue was best for the finest changes. A. M. C.

**Jacottet, G.** Étude sur les altérations des cellules nerveuses de la moelle et des ganglions spinaux dans quelques intoxications expérimentales. *Beitr. zur path. Anat. u. zur allgem. Path.* **22**: No. 3, pp. 443-465, 9 fig. *Abstr. Zeitsch. f. wiss. Mikros.* **15**: 3; 1897.

The author applies Nissl's method with modifications of Schaffer, Pandi, Sarbó, Sadowsky; the last being especially useful. This method is to harden pieces of fresh cord in an

aqueous 10 per cent. solution of formalin for two to four days; putting it in 95 per cent. alcohol for forty-eight hours, and finally in absolute alcohol for an indefinite time. If sufficiently hard, pieces are cut directly or in celloidin. Sections are stained one to two minutes in a concentrated solution of fuchsin in 5 per cent. carbolic acid aqueous solution. After staining, sections are bleached in an acetic acid solution till the differentiation between the white and gray matter is clear; further differentiation occurs in the absolute alcohol, and sections are cleared in xylol and mounted in balsam. Sadowsky used methylen blue instead of fuchsin, a freshly prepared 5 per cent. solution, in which the slides remain for half an hour. Ramón y Cajal's excellent method was used: passing sections direct from 95 per cent. alcohol in concentrated aqueous solution of thionin for a few minutes, and then into a mixture of equal parts of anilin and absolute alcohol; when the sections only showed a light blue they were put in xylol and then balsam. These methods, however, do not produce permanent stains. A. M. C.

**Coles, A. C.** The Blood: How to Examine and Diagnose its Diseases. London (Churchill), 1898. 8; 60 plates.

**Ogneff, J.** Ueber die Entwicklung des elektrischen Organs bei Torpedo. *Arch. f. Anat. u. Phys. Phys. Abth.* p. 270, 1897. *Abstr. Zeit. f. wiss. Mikro.* **15**: 1898.

**Jolly.** Recherches sur la valeur Morphologique et la Signification des Différents Types de Globules blanc. Thèses. Paris, 1898.

**Birmingham, A.** A Study of the Arrangement of the Muscular Fibers at the Upper End of the Esophagus. 5 fig. *Transact. of the R. Acad. of Med. of Ireland.* **16**: pp. 422-431 and 432-440.

**Brouha, M.** Recherches sur le Développement du Foie, du Pancréas de la clison mésentérique et des Cavités hépato-entérique chez les Oiseaux. 3 taf u. 20 fig. *Journ. de l'Anat. et Phys. T.* **34**: 305-363.

A PERFORATED SWIMMING PORCELAIN CUP FOR WASHING OUT SPECIMENS. — The accompanying illustration shows a porcelain cup which is perforated and is closed by a cork. By the aid of the cork the cup swims in the water. It is made for washing out specimens, e. g., pieces which have been decalcified.

EMIL AMBERG, M. D., Detroit, Mich.



## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAIRE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review  
to H. H. Waite, 710 East Catherine street,  
Ann Arbor, Michigan.

**Opitz, E.** Beiträge zur Frage der Durchgängigkeit von Darm und Nieren für Bakterien. Zeitschrift. f. Hyg. 29: 505-552, 1898.

The apparently positive results obtained by numerous investigators along this line, the author believes, are due to

faulty technique, failure to make a histological examination, and delay in making cultures after the death of the animals used for experimentation. To obtain data as to whether bacteria invade other parts of the body through penetration of the intestinal wall, rabbits were used for experimentation. Solutions of sodium arsenite, tartrate of antimony and cantharidin were used to produce death, which resulted in from three and a half hours to five days. Plate cultures were made in gelatin and agar from the kidney, liver, bile, heart, blood, spleen, mesenteric glands, and ascitic fluid. Cultures made from the spleen, bile, liver, and blood remained practically germ free, while cultures from the mesenteric glands, kidney, and urine contained many colonies of the bacillus coli communis. The bacteria found in the kidney, urine, and mesenteric glands cannot be explained through transportation by the blood current, as the spleen remained germ free. The explanation of their appearance is through direct penetration of the intestinal walls, and, as some of the animals were not examined for some hours after death, the invasion was probably post mortem.

In order to ascertain whether bacteria circulating in the blood penetrate the kidney epithelium, dogs were used and the urine excreted by them was examined. Filtered and unfiltered suspensions of both pathogenic and non-pathogenic bacteria were introduced into the carotid artery and the jugular vein. Colonies were obtained from the urine in great numbers when voided shortly after the introduction of the suspensions. Histological preparations of the kidney showed extensive hyperæmia of the glomeruli, and hence the appearance of bacteria in the urine was probably due to pathological changes.

The author sums up the results of his work as follows: The normal intestinal wall is impenetrable for intestinal bacteria. A passage of bacteria into the chyle does not take place during digestion. Slight alterations of the intestinal wall cannot remove its protective power. Severe mechanical and chemical lesions only rarely lead to an invasion of the circulation by bacteria. An agonal penetration of germs in the circulation is, at least through the intestinal wall, not proven. A physiological excretion of bacteria in the blood through the kidneys does not take place. The frequently observed appearance of germs in the urine shortly after injection into the circulation is due to chemical and mechanical lesions of the vessel walls and kidney epithelium.

H. H. W.

Hill, H. W. Branching Forms of the Bacillus Diphtheriae. Jour. Boston Soc. Med. Sci., Jan. 17, 1899

The author of this paper has found that branching forms of the diphtheria bacillus occur in more than 50 per cent. of all positive diphtheria cultures which he has investigated. He thinks it is probable that the ordinary diphtheria bacillus represents a variation or degeneration of a higher type of plant than the bacillus. Most frequently the branching resembles that of a streptothrix; at times, however, that of a cladothrix. He suggests that possibly the branching is due to some peculiar condition of the external layers of the cell, which may be due to degenerative changes, and hence the branching is only apparent. The latter explanation he thinks improbable.

Cultures which contained the branching forms were tested by experimentation on guinea pigs, all of which died within two days, showing lesions similar to those produced by inoculation of ordinary diphtheria cultures. Cultures from the dead animals on blood serum again showed branching forms. Branching did not occur in the living guinea pig nor on normal tissues from the guinea pig inoculated with pure cultures.

H. H. W.

Otolenghi, D. Ueber die Widerstandsfähigkeit des Diplococcus lanceolatus gegen Austrocknung in den Sputa. Centrblt. f. Bakt. 25: 120-122, 1899.

The experiments were made with pneumonic sputa received from three patients on the fourth or fifth day of the disease. The sputa were spread on linen and allowed to dry in diffuse light at a temperature of from 15 to 20 degrees C. Cultural and inoculation experiments were made at varying periods, inoculation as a means of determining the duration of virulence, cultural to determine the duration of vitality of the diplococcus.

The first sample of sputum maintained its virulence after thirty-six days exposure to dessication; its vitality for sixty days. Both the virulence and vitality of the second sample were unimpaired for seventy days. The third sample was virulent for sixty-five days, its vitality lasting for more than eighty days. These experiments show that the diplococcus in dried sputum is capable of maintaining its virulence for more than seventy days, and its vitality for a still longer time. The observation was also made that the sputum, which was thin and frothy, dried in scales which were easily scattered by the air, and in this way the germ was widely distributed. The above observation indicates the necessity of rigid disinfection of pneumonic sputa.

H. H. W.

Hill, H. W., Dr. A Method of Preparing Test Objects for Disinfection Experiments. Trans. Amer. Pub. Health Ass'n. 24: 1-4; 1898.

This is a modification of Koch's method of drying organisms to be tested, on silk thread. A glass rod with cotton wrapped around the end is placed in a test-tube in such a way that the cotton forms a stopper for the tube, and the whole thing is sterilized by dry heat. The sterilized rod may be dipped in the preparation to be tested—an aqueous emulsion sticks best to the glass—or it may be rolled on the surface of a solid culture, taking up a thin film which dries quickly. The rod may then be returned to the sterilized tube or placed in a culture medium. The rods are easily labeled and exposed and are less difficult to sterilize than silk threads.

E. M. B.



## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Pathological Laboratory, Boston City Hospital, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

## CURRENT LITERATURE.

**Krompecher.** Beiträge zur Lehre von den Plasmazellen. Ziegler's Beiträge, 24: Heft. 1  
Krompecher agrees with those who consider the plasma cells to be infiltration cells, and derived from lymphoid cells, but also thinks that a larger form may possibly be derived from multinuclear leucocytes. He considers the presence of plasma cells to indicate a progressive activity. He has seen all stages of transition between plasma cells and epithelioid cells, and thinks that plasma cells are concerned in the formation of connective tissue.  
R. M. P.

**Fuerst.** The Changes Produced in Epithelium by Moderate Degrees of Heat and Cold. Beitrage zur Path. Anat., 1898.  
Fuerst, working under Ribbert, describes the following changes in the ears of rabbits and guinea-pigs after freezing lightly with ether spray, or using water at about 50 C. The epidermis is increased to eight times its normal thickness by an increase in the size of the cells and by the formation of new cells. Many giant cells, formed by multiple direct division of single cells, appear among the epithelial cells. Regeneration of tissue from an injury, produced after exposure to heat or cold, takes place three times as rapidly as normally. Slight chemical irritation produces similar proliferation, but no giant cell formation. He considers the rapid proliferation to be due not to any peculiar action of the thermal or chemical irritant, but to excessive regeneration. His explanation of the formation of giant cells is interesting. He holds that by the thermic irritation the protoplasm of the cell is injured, while the nucleus is intact. The injury to the protoplasm prevents true karyokinesis, but allows unlimited direct nuclear division.  
R. M. P.

**Thomas.** Med. and Surg. Reports of the Boston City Hospital, 1899.  
Thomas reports a case of the formation of true bone in the brain, resulting from the presence of *Coccidium oviforme*. The tumor had caused no symptoms during life. At the autopsy a small calcareous mass was found in the anterior central convolution, which was atrophied. This mass, on microscopical examination, was found to consist of true bone, with laminations, lacunæ, and canaliculi, containing marrow spaces, with œdematous connective tissue, and occasional large granular connective tissue cells (fat granule cells). Outside of the bone was a layer of increased neuroglia tissue, a secondary gliosis. Near the nodule were also seen long, spindle-shaped spaces surrounded by connective tissue cells, and giant cells, the whole resembling the granulation tissue sometimes seen around fat crystals. In the center of the nodule was a large space filled with granular detritus in which were many small oval bodies. These bodies, the coccidia, had a distinct capsule, occasionally double, and were either empty or filled with granular material.  
R. M. P.

# Journal of Applied Microscopy.

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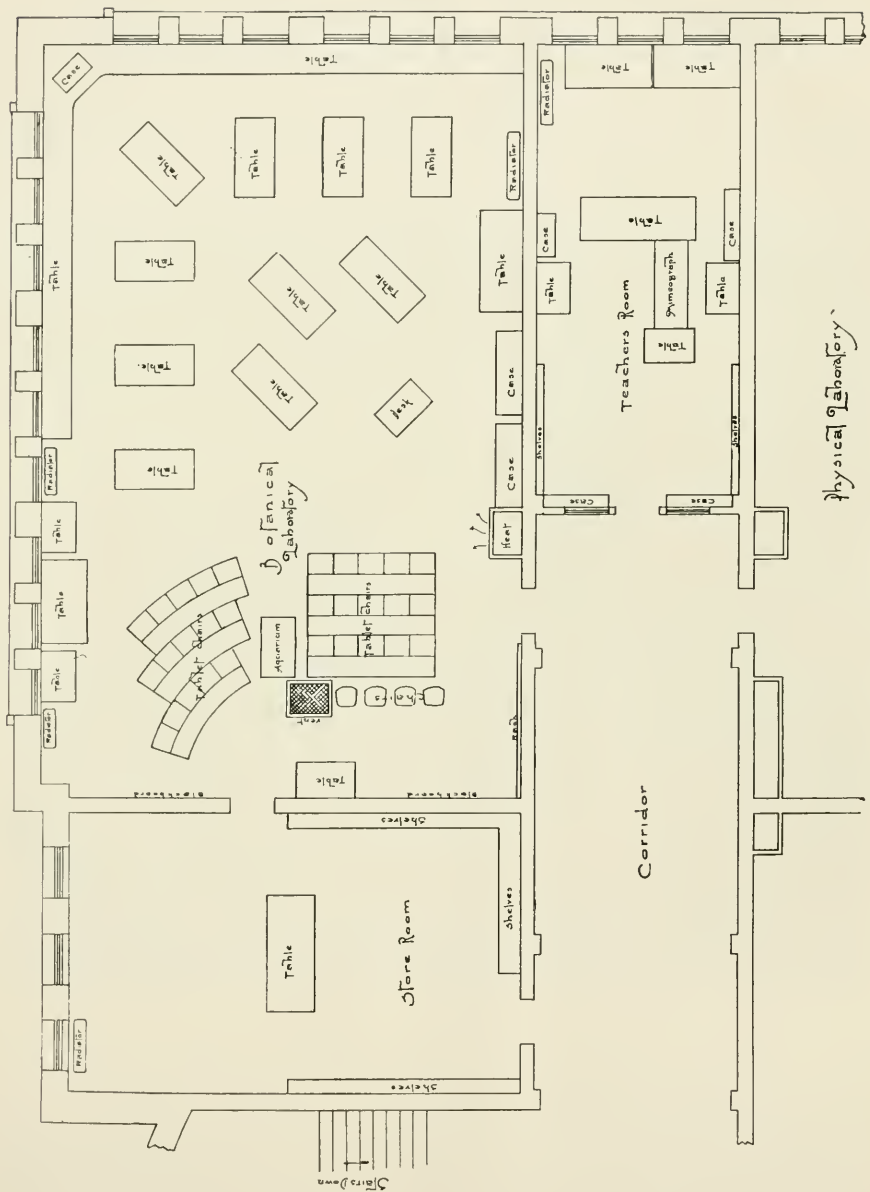


DULUTH HIGH SCHOOL.

## Laboratories of the Duluth High School.

The excuse for this article is two-fold: first, to give some idea of a high school laboratory that was put into a room not especially designed for the purpose, and of the nature and scope of the work done there; second, to make a modest plea for more laboratories and better equipments for the high schools throughout the country.

The room in question is on the third floor of what might be called the north corner of the building. One end, the northeast, and one side, the northwest,



Physical Laboratory

PLAN OF THE BOTANICAL LABORATORIES, DULUTH HIGH SCHOOL.

look out upon the yard. It is unobstructed by the proximity of other buildings or trees. Adjoining one side of this laboratory is a small room for specimens and work materials; it serves also as a sort of private room for the teacher. At one end of the laboratory, and between it and the stairway, is a dimly-lighted store room; thus it is isolated from the noise and bustle of the school in general, and is almost ideally located for a laboratory. The dimensions and floor space of these rooms are as follows:

Laboratory, . . .	53 x 32	feet, making a floor space of 1696	square feet.
Store room, . . .	21 x 19.5	" " " "	409.5 "
Teacher's room,	31 x 14	" " " "	434 "



GENERAL VIEW OF LABORATORY.

1014 square feet of the laboratory floor space is taken up with the laboratory proper, and 618 square feet by tablet chairs used for recitation purposes.

There are fifteen windows in the room, nine on the side and six on the end. These are 30 inches by 86 inches, making 285 square feet of lighting surface. They are 39 inches from the floor, or about the height of the students' shoulders as they sit at the tables.

The room receives no direct sunlight during school hours. The ceiling, which is 22 feet high, and the walls, are white, making a very evenly diffused, soft light, exactly suited for the purpose for which it is used, and sufficiently strong for the use of a microscope in any part of the room.

The tables are neither elaborate nor expensive, and are not specially constructed laboratory tables, but they are of solid oak, 36 x 67 inches, and



29 inches high, filled and oiled, with four shallow drawers for paper, pencils, slides, etc. Across the northeast end and along two-thirds of the northwest side of the room, is a plain oak wall-table twenty-four inches wide, supported by brackets, and of convenient height for work when the students sit on stools twenty-eight inches in height. The other tables, nine in number, accommodate four students each, and are placed in convenient positions with regard to the light; six are placed with the end to the wall table, but with a sufficient passage-way between. The other three are similarly placed, but nearer the center of the room. There is also a plate glass aquarium 16 x 16 x 27 inches, placed well toward the south-east end of the room.

On account of the number of students, it is necessary to have them work by



DETAIL OF WORK TABLES AND ARRANGEMENT.

twos. The wall table accommodates fourteen students. The lower tables accommodate thirty-six students, who receive their light from the upper part of any convenient window, and from over the heads of those working at the wall table. This, owing to the height of the windows, is a very economic apportionment of space and light, and is very satisfactory. On the side of the room opposite the windows are a radiator, a large reference table 35 x 84 inches, two oak cases 148 inches high, 66 inches wide, and 20 inches deep, with glass doors and six drawers, a ventilating shaft, and a hat and cloak rack where students who work after hours may leave their wraps. The remainder of the other side of the room not occupied by the wall table is devoted to plant boxes, a general experiment table, and radiators. At the southwest end of the room are a blackboard, and a door that opens into the store room.

Each two workers are provided with a compound and a dissecting microscope, a box containing scalpel, scissors, and forceps, a set of six reagent bottles, watch glasses, bottles and slides, necessary for work. Students are required to furnish pens, pencils, razors, paper, and note books. Each student is made individually responsible for all apparatus used, and is required to keep the same, with table, clean and in good order. The result of thus imposing this responsibility is, that although the students have almost unrestricted access to the laboratory for six days in the week, there is seldom anything taken or misplaced. A sense of responsibility soon begets in the students a pride in an orderly, well appointed work-room, in which they spend a year of consecutive daily work.

The time given to the subjects of botany and zoölogy is not all that could be desired, there being only half a year devoted to each. Botany begins with the second semester of the second year, and zoölogy with the first semester of the junior year.

The two kingdoms are taken up similarly, so that what is said of one, in regard to method, will apply to the other. In botany, the course is planned to cover the entire plant kingdom, not exhaustively, of course, but yet in a systematic and scientific manner. The different natural groups are taken up separately, and studied as to their classification, structure, and relation to other groups, with some work in physiology.

The weekly programme (subject to some variation) is as follows: Monday, a talk or lecture by the teacher, bearing on the group as a whole, on classification, and a general outline of the group to be studied, in which blackboard and wall charts are used; Tuesday, Wednesday, and Thursday, two hours each day are spent in laboratory work on some typical specimen of the group under consideration; Friday is devoted to a quiz or written work covering the lecture and laboratory work of the week.

The writer has also prepared a series of lantern slides representative of each group of plants and animals studied, embracing many forms not obtainable for laboratory work. These are generally used after the study of a group, to give a general review and more comprehensive idea of the whole subject. For this purpose a good stereopticon, with which may be used either sunlight or an electric arc, is always in readiness, and can be used without special preparation. This is found very helpful, and amply repays the great amount of labor and time expended in the making of lantern slides, etc. Thus, the work of the week consists of a sort of pre-view laboratory work, a summing-up, and a general review after each group studied.

In the last decade, school and college curricula have undergone a gradual but marked evolution. People in general demand more and more training in science, some because they believe it to be what is termed "practical education," others because they are satisfied that it is the best, and prefer that the mind shall be trained by these natural laws. Whatever the reason may be, the demand certainly exists, and recitation rooms and text-books must give way, to some extent at least, to laboratories.

It is not infrequent, however, to find a subject in science, or even a "scientific course," introduced into the curriculum, where no laboratory at all has been

provided, and where no apparatus is available. Those who have charge of these things either do not know, or frequently forget, that work in science without a laboratory and apparatus is as impossible as work in manual training without a shop or tools; and that text-book science is only on a par with text-book manual training, or text-book blacksmithing.

The ordinary text-book course in science is about as fruitless a waste of time as can well be imagined. It may have some value in a literary way, but it certainly *is not* and *should not be* paraded as science. I have no fault to find with a good text-book; it is the way it is misused, or over-used, of which I complain.

The basis of all science work should be knowledge at first hand—individual investigation—"something acquired"—not "something learned." I believe it is the best, and practically the only way during school life, of training the senses, the avenues through which all knowledge must enter. It should also constantly exercise the judgment on original problems. "Text-book science is a make-believe, a misnomer, and is better out of a course of study than in it. It gives no special mental training, and adds no knowledge worth having."

I believe that the foundation of every course in general botany or zoölogy should be some good, plain, natural system of classification, around which all laboratory and reference work should be gathered. It will form a foundation for future reading and reference. It is the classification of knowledge, without which all knowledge loses half its value.

Then if a natural classification is to be the basis of our course, it is highly necessary that the various orders and classes be taken up in a somewhat logical order, and that types enough be introduced and studied, not only to fix the classification, but to make apparent the more common likenesses and differences.

To teach botany or zoölogy intelligently, or to study the animal or plant kingdoms from the standpoint given, without the use of the microscope, is not only impracticable but impossible. By far the greatest number of animal and plant forms cannot be studied without the aid of the microscope, to say nothing of its necessity in the study of the anatomy and histology of the higher and more complicated forms.

It is sometimes urged that we do not need a microscope for the study of the common forms around us. This statement comes from an ignorance of what is around us, and what is common. I would not convey the idea that an object, to be of any interest or value, must be viewed through a microscope, but I do mean to say, and to insist, that there are many important forms which have their place in the animal or plant kingdoms, and which are of the greatest importance in the economy of nature, that must be so studied, if studied at all.

The microscope is no longer a plaything—an instrument for pastime and amusement. It is a piece of apparatus for actual scientific work. It is no longer viewed as the thing to be studied—it is simply a means to an end, as is a pen or a hammer. It is as indispensable to good work in science as are tools to good work in carpentry.

Since a course in botany or zoölogy should give an idea of the range and extent of the various life forms, with a fair acquaintance with at least a few of the type specimens, and their most evident relations, it is clear to anyone who



has undertaken the task that the microscope is necessary in many ways, not only on account of the minute forms, but on account of the material that can and must be collected and preserved.

The smaller forms may be brought into the laboratory during the summer and fall months, and kept in a growing state or in preservative, and used during the months when outdoor work is impossible and outdoor material is not available. In fact, I do not see how even a half year can be profitably spent in botany (especially in the northern parts of the country) without the free use of the microscope and laboratory methods.

Formerly the high price of microscopes and the difficulty of importing them were serious drawbacks to their general use. Happily this is no longer the case. We make several first-class microscopes in this country, especially adapted to the needs of high schools, and at very reasonable or even low prices. In fact, microscopes are cheaper than text-books, and tables cheaper than school desks.

The microscopes in my laboratory have been in almost constant use for six years, and are as good as new, and it is safe to estimate that they will last four or five times as long as they have been in use. They are each used daily by from four to six students. The cost of text-books for the same number of students for the estimated time that the microscopes will last, will aggregate more than the cost of the microscopes. While we may thus compare the cost of equipments, no comparison between the value of the results of the laboratory and text-book methods can be made.

There are but two places to study science—the field and the laboratory; and a cardinal principle is, to permit nothing to come between the student and nature. Whatever does this, whether text-book, chart, or teacher, is a hindrance. In a course of botany or zoölogy, the laboratory work forms the root of the course, the text-book and lecture the leaves.

A properly equipped laboratory is becoming more and more a necessity. As specially trained and competent teachers are sent out by our colleges and universities, the work in structure and classification is not only delegated to, but demanded of the high schools, as preparatory to the more advanced work of physiology and morphology—the basis of the college course. In addition to this demand, some of the states make appropriations of stated amounts to high schools, or pay for a per cent. of apparatus purchased. It is to be hoped that more states will give like encouragement to their high schools, and that we in the states that have given this encouragement will show our appreciation of the facilities afforded us, and endeavor to effect a marked advancement in thoroughness and excellence of the work done.

A. J. WOOLMAN.

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DR. HUGH M. SMITH announces that on June 1st, a new marine biological laboratory, equipped for investigators, will be opened at Beaufort, N. C., by the U. S. Fish Commission. The laboratory will be under the direction of Dr. H. V. Wilson, professor of biology at the University of North Carolina. The Fish-Hawk will have its headquarters at Beaufort in the early fall, during a biological and topographical survey of the oyster grounds of the state.



## An Improvement in the Technique of Making Blood-Serum Culture Media.

The usefulness of coagulated blood-serum, with or without the addition of glucose bouillon (Loeffler's blood-serum mixture), as a general culture medium for the propagation of pathogenic bacteria, makes its easy manufacture a great desideratum. The original "slow" method, involving the use of special apparatus and a great expenditure of time, has now been abandoned in most laboratories for the more satisfactory Councilman-Mallory method. While a great improvement over the previously employed method, still most workers will agree that even this procedure is open to certain objections, chief among which are the necessity for more or less careful supervision of the process while the medium is undergoing coagulation and the occasional complete destruction (or at least the making of an unsightly product) by the bubbling which results from the accidental production of too high a temperature where the ordinary hot-air or steam sterilizer is used in lieu of the special coagulating apparatus.

For somewhat over a year I have employed a device which obviates these and certain lesser difficulties, and makes the manufacture of slants of coagulated blood-serum, or Loeffler's medium a procedure which is now undertaken with the assurance of securing a perfectly satisfactory product at an expenditure of labor not greater than is required for making a batch of nutrient agar and essentially without the possibility of anything going wrong if the details are accurately followed. This result is attained by the simplest possible device and with the employment of only such apparatus as is found in even the most modest laboratory. In addition to obviating the difficulties above mentioned, this method possesses marked advantages in the ease of sterilization of the medium and in its keeping power.

The blood is collected at the slaughterhouse in the usual manner, museum jars of about four liters capacity, with clamped tops, being the best receptacles. The jars are merely washed thoroughly and no attempt is made at rendering them perfectly sterile. About half an hour after the blood is collected a clean glass rod is passed around between the clot and the side of the jar, breaking up any adhesions between the two. The jar is then left in a cool place for not less than twenty-four hours, until the clot is quite firm, when the serum is removed by means of a pipette with a bulb blown in it to increase its capacity and with a soft rubber tube attached. During this step, and indeed up to the point where the serum is poured into the test-tubes, no special aseptic precautions are taken, mere cleanliness being all-sufficient.

The serum is now run into test-tubes which have been previously sterilized by dry heat, using for this purpose a filling funnel or, for small quantities, a 50 cc. burette. If an ordinary small funnel without special attachment is used, the entrance of air bubbles is inevitable and these remain entangled in the surface of the serum for some hours, giving rise to an unsightly product if the serum is coagulated before they have completely disappeared. If Loeffler's

medium is to be made, one part of a one or two per cent. glucose bouillon is added to every three parts of blood-serum before tubing.

After filling all the tubes which it is desired to prepare, the remaining portion of the liquid serum may be kept for future use by pouring it into bottles and adding a sufficient amount of chloroform to form a layer of 2 mm. to 3 mm. on the bottom. The bottle is then closed with a tightly fitting cork (*not with a cotton plug*), thoroughly shaken, labeled, and set aside. This will preserve the serum

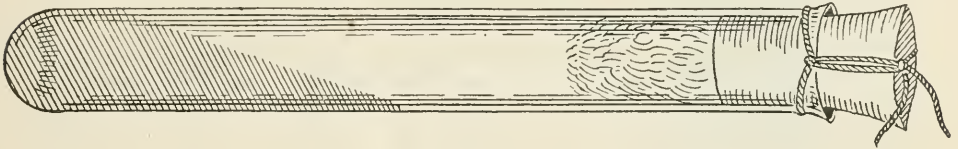


Fig. 1.

indefinitely, and it is available for use at any future time by vaporizing the chloroform.

To return to our tubes: after filling, the next step is the one to which I desire to direct special attention, as careful observance of this point obviates all difficulty connected with solidifying the serum without bubbling. A number of corks of the best quality, and *one or two sizes larger than will easily go into the mouth of the tube*, are put into a wire basket and steamed in the Arnold sterilizer for at least twenty minutes. When taken out they will be both sterilized and so softened that they can readily be forced into the tubes for some distance. The cotton plug of each test-tube is then burned off to a level with the top of the tube and the cork shoved well in, care being taken to avoid contamination of the cotton and cork during this procedure. Then, by means of a fairly stout piece of twine, each cork is tightly tied into its tube, using the knot commonly employed by druggists for this purpose (Fig. 1). A lipped test-tube (the usual kind) must be employed. The tubes used by the New York and other health departments will not answer.

After tying in all the corks, the tubes are placed on the proper slant in a receptacle which can easily be made from a cigar box and a piece of wire gauze such as is used for fly screens. No description of this apparatus is necessary, as its construction is well shown in Fig. 2.

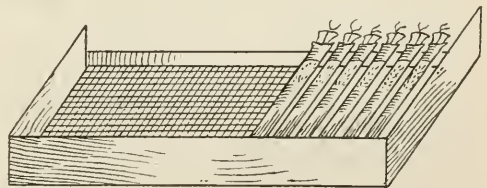


Fig. 2.

Having filled the box, preferably with a single layer of tubes, a wire basket, such as is found in every bacteriologic laboratory, is placed upright in the Arnold sterilizer, and upon this is laid another, flat. On the latter is placed the box of tubes. This should bring the tubes about to the level of the top of the sterilizer, where they can be easily observed, and where, in the method used, the temperature falls just short of 100° C. A towel, of not too close weave, is then thrown over the sterilizer. *This should be the only cover, both inner lid and outer jacket of the sterilizer being dispensed with* (Fig. 3). The

gas is then started under the sterilizer, and if the corks have been tightly shoved in and firmly tied no watching of the serum is required.

Within from ten to twenty minutes from the time of getting up steam (according to the amount of serum in the tubes, the size of the gas flame, and the composition of the mixture—pure blood-serum or Loeffler's medium), the serum will be found coagulated evenly and without any disfiguring bubbles.

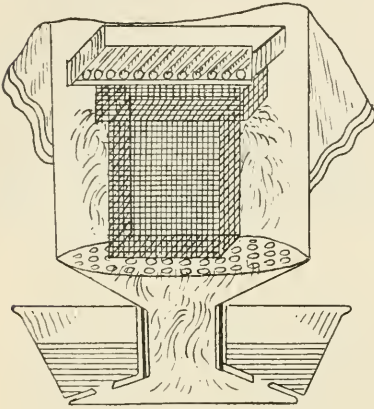


Fig. 3.

The mode of action of the cork is simple. By it, air of room temperature is closely confined in the tube. As it becomes heated, this air, tending to expand, increases the pressure in the tube, and hence it is impossible for boiling to take place at 100° C., the temperature of the sterilizer.

The tubes should be left in the sterilizer for half an hour, in order to insure thoroughness of the first sterilization. Just here is another advantage of the cork.

Without it prolonged action of heat dries out a portion of the serum along the thin slanting edge. With it this occurs to only a very insignificant extent even if left in the sterilizer for a considerable time. The tubes are similarly steamed on the two subsequent days, after which they may be put aside and will keep indefinitely. I have now on hand slants of Loeffler's blood-serum mixture made eighteen months ago and they are still in perfect condition, the cork preventing evaporation.

Where a large number of tubes is required, a wire apparatus could be easily constructed wherein several tiers of tubes could be arranged on the proper slant, but this I have never found necessary; for the process, after the tubes are properly plugged, requires no special skill, and even the laboratory boy can be trusted to remove one batch and place another in the sterilizer.

To summarize the advantages of the method above described :

1. A perfectly smooth culture surface is secured without the necessity of close supervision during coagulation.
2. Coagulation is accomplished in from ten to twenty minutes.
3. The medium does not become dried out during the processes of coagulation and sterilization.
4. Sterilization is conducted at a high temperature, thus insuring thoroughness.
5. The culture medium does not subsequently dry out, but may be kept indefinitely.

The only disadvantage I have found connected with the method is that a tube with a cork and a deep-seated cotton plug is not so easy to manipulate when making cultures as is one closed in the usual way. This inconvenience is slight and does not weigh against the many advantages.

It is well when going to the slaughter-house to obtain a sufficient quantity of

blood to last for many months. By the addition of chloroform (in the manner above mentioned) that portion of the serum which is not at once used can be preserved indefinitely. When it is desired for use, the chloroform is driven off by placing the serum in a large flask in a water bath, which is kept at a temperature lower than is required to coagulate albumin. I have found 58° C. about right. The passage of a current of air through the serum while in the water bath would materially hasten this step (which ordinarily takes several hours), but it is impracticable on account of the viscid nature of the material, owing to which the current of air soon converts the serum into a mass of bubbles which completely fill the flask and pass out through the aspirating tube. Transferring the serum to a clean flask about every twenty minutes will somewhat hasten this process. Freedom from the characteristic odor of chloroform indicates its complete evaporation, and the serum should not be used until this point is reached. Further manipulation of the chloroform-preserved serum is identical with that employed with the fresh product.

ERNEST C. LEVY, M. D.

Bacteriologic Laboratory, Medical College of Virginia.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

### III.

#### KILLING AND FIXING AGENTS.

In this short account, only the reagents which are at present considered most valuable for botanical work will be considered. Probably no process in microtechnique is in more urgent need of improvement than this first step of killing and fixing. Nearly all of our formulæ are merely empirical, for very few botanists are expert chemists, and those who have the requisite knowledge of chemistry are interested in physiological problems rather than in microtechnique. The principal ingredients of the usual killing and fixing agents are: alcohol, chloroform, chromic acid, acetic acid, osmic acid, formic acid, picric acid, sulphuric acid, platinum chloride, corrosive sublimate, and formalin. We shall consider first

#### THE ALCOHOLS.

*a. 95 per cent. Alcohol.*—This is in quite general use for material which is needed only for rough work. It is extremely convenient since it kills, fixes, and preserves at the same time and needs no changing or washing. It really has nothing to recommend it for fine work. It causes protoplasm to shrink, but cell walls usually retain their position, so that 95 per cent. alcohol will do for free-hand sections of wood and many herbaceous stems, but even free-hand sections of tender stems, like young geraniums and begonias, will look better if better reagents are employed. Alcohols weaker than 95 per cent. are not to be recommended as fixing agents, although 70 per cent. alcohol or even 50 per cent. will preserve material for habit work.

*b. Absolute (100 per cent.) Alcohol.*—This is a good killing and fixing



agent, but is rather expensive. It causes but little shrinking of the protoplasm and is a time saver if material is to be imbedded in paraffin. With 95 per cent., or with absolute alcohol, objects are generally left in the reagent until needed for use, but such material becomes very brittle. The addition of glycerine is an improvement if material is to be kept long. Acetic acid has been used with alcohols to counteract the tendency to shrink. One of the most successful of the alcohol combinations is

*c. Carnoy's Fluid.*—Absolute alcohol, 6 parts; chloroform, 3 parts; glacial acetic acid, 1 part. The penetration of the reagent is excellent, and only a few hours are needed for fixing. Material should be washed in absolute alcohol (perhaps 95 per cent. alcohol would do no harm) until there is no odor of acetic acid. This should not require more than one or two hours. It is better to imbed in paraffin at once, but when this is not convenient the material may be transferred to 85 per cent. alcohol and then to 70 per cent., where it may be left until needed. Cyanin and erythrosin, fuchsin and iodine green, and similar combinations give particularly brilliant staining after this reagent.

#### THE CHROMIC ACID GROUP.

Chromic acid, or solutions with chromic acid as a foundation, are the most generally useful killing and fixing agents yet known to the botanist. A 1 per cent. solution of chromic acid in water gives good results, but it is better to use the chromic in connection with other ingredients, such as acetic acid, formic acid, osmic acid, etc. The proportions of the various ingredients must, for the present at least, be determined by experiment. With favorable objects like fern prothallia, spirogyra, and other things which can be watched while the fixing is taking place, suitable proportions are rather easily determined because specimens, after being placed in the reagent, may be examined at frequent intervals, and combinations which cause plasmolysis may be rejected and different proportions tried until satisfactory results are secured. For example, fern prothallia might be placed in the following solution: chromic acid, 2 gr.; acetic acid, 1 cc.; and water 97 cc. If plasmolysis takes place, weaken the chromic or strengthen the acetic, since the chromic has a tendency to produce contraction, and the acetic to cause swelling. Too large a proportion of acetic acid, however, may cause distortion, and hence it would be better to weaken the chromic. In case of fern prothallia, 3 parts chromic, 1 part acetic, and 396 parts water will cause practically no plasmolysis, and the fixing is sufficiently thorough to permit imbedding in paraffin. A combination may be quite satisfactory for fern prothallia and still fail to give good results with spirogyra. For very critical work the most favorable proportions must be determined for the particular plant under investigation. When the effect of the reagent cannot be observed directly, it is well to make a free-hand section and thus determine whether plasmolysis is taking place. It is not safe to judge the action of a fixing agent by the appearance of sections cut from material which has been imbedded in paraffin, because shrinking of the cell contents often takes place during the transfer from absolute alcohol to the clearing agent or during infiltration with paraffin, and sometimes during even later processes. When in doubt as to proportions we would suggest 2 cc.

chromic acid, 2 cc. acetic acid, and 296 cc. water as a good formula for most purposes.

The time required for fixing undoubtedly varies with different plants, but twenty-four hours may be considered a minimum even for the most delicate objects. It is well known that zoölogists allow fixing agents like Müller's fluid and Erlicki's fluid to act for weeks before the material is passed on to the next stage, and it may well be questioned whether botanists have not made a mistake in allowing the chromic solutions to act for so short a time. At present most botanists recommend sixteen to twenty-four hours for material which is to be imbedded in paraffin, but some recent experiments in my laboratory indicate that material which has been in the fixing fluid for two or three days is better able to withstand the subsequent processes. More rapid penetration and consequently more immediate killing can be secured if the reagent is kept warm (30 degrees to 40 degrees C.) Since chromic acid has a tendency to render objects hard and brittle, it is often better to use some other fixing agent if much difficulty is anticipated in the cutting.

After fixing is complete, the reagent should be washed out with water. Running water is desirable, and where this is not convenient the water must be changed frequently. Any material should be sufficiently washed in sixteen to twenty-four hours, but the time may be shortened about one-half by using lukewarm water.

Some of the formulæ are as follows :

a. *Strong Chromo-acetic Solution*.—1 gr. chromic acid, 1 cc. glacial acetic acid, 98 cc. water. This solution has been used quite extensively in embryological work upon the higher plants.

b. *Weak Chromo-acetic Solution*.—(Schaffner's formula): 0.3 gr. chromic acid, 0.7 cc. acetic acid, 99 cc. water. This has also been used in embryological work. It causes little or no plasmolysis, but the chromic seems rather weak. Difficult material, like Aster heads and ripe *Capsella* pods, cuts more readily after this reagent than after the stronger solution.

c. *Flemming's Fluid*.—(Weaker solution.)

A. 1 per cent. chromic acid, 25cc.

1 per cent. acetic acid, 10cc.

Water, 55cc.

B. 1 per cent. osmic acid, 10cc.

Keep the mixture A made up and add B, as the reagent is needed for use, since it does not keep well. This fluid is quite expensive on account of the osmic acid. For cytological work it gives better results than any other fixing agent which has yet been thoroughly tested. It is especially recommended for chromosomes, centrosomes, achromatic structures, and mitotic phenomena in general. Material should be in very small pieces, one-eighth of an inch square or in thin slices one-eighth of an inch or less in thickness, for the fluid penetrates poorly. The blackening due to the osmic acid may be removed by peroxide of hydrogen just before the slide is passed from the alcohol into the stain. Flemming's safranin-gentian violet-orange combination gives its most brilliant results after this reagent.

\* *d. Merkel's Fluid.*—Equal volumes of a 1.4 per cent. solution of chromic acid and a 1.4 per cent. solution of platinic chloride. This is also an expensive reagent. It is recommended for mitotic phenomena, but does not seem to equal Flemming's solution.

*c. Hermann's Fluid.*—

1 per cent. platinic chloride, 15 parts.

'Glacial acetic acid, 1 part.

2 per cent. osmic acid, 4 or 2 parts.

This is the most expensive fixing agent yet discovered, and for botanical purposes it does not seem to be any better than the cheaper chromic mixtures. It is mentioned here with chromic mixtures because it originated as a variation of Flemming's fluid, the platinic chloride being substituted for the chromic acid.

According to Lee, the chief objection to all mixtures into which chromic acid enters is that "it precipitates certain of the liquid albuminoids of the tissues in the form of filaments or network, which are often of great regularity and simulate structural elements of the tissues." Nevertheless, the mixtures which have just been described are the best which have yet been thoroughly tested. If material killed in any of the above mixtures is not well washed, the hæmatoxy-lins will not stain. It is claimed that the anilins will stain in spite of poor washing, but it is a question whether such preparations are as permanent as those from well washed material.

#### PICRIC ACID.

Use a saturated solution in water or 70 per cent alcohol. One gram of picric acid crystals will saturate about 75 cc. of water or alcohol. This reagent penetrates well and does not make the material brittle. It is to be recommended when difficulty is anticipated in the cutting. If used cold, the time varies from one to twenty-four hours, depending upon the character of the tissue and size of the specimen. If used hot (85 degrees C.), five or ten minutes will be sufficient. Material should be washed in 70 or 50 per cent. alcohol. Water is injurious, and some even go so far as to avoid aqueous stains unless the material has been thoroughly washed. The washing should be continued until the material appears whitish, and the alcohol no longer becomes tinged with yellow. Picro-carmin gives its best results after this reagent. Picric acid can be combined with various other fixing agents, and so we have picro-sulphuric acid, picro-nitric acid, picro-chromic acid, picro-chromic-sulphuric acid, and picro-osmic acid.

#### CORROSIVE SUBLIMATE.

Use a 2 to 5 per cent. solution in water, or 70 per cent. alcohol. The addition of about 1 cc. of glacial acetic acid to 100 cc. of this solution is certainly an improvement. The time required is considerably shorter than for chromic solution. From one to ten hours will be found to be sufficient. If used hot (85°C.) only five or ten minutes is required. Washing may be done with water, but 50 per cent. alcohol is better. If a few drops of an iodine solution be added to the alcohol, the alcohol takes on a brownish color, but soon clears up. If the addition of iodine be continued, the washing is complete when the alcohol no longer clears up, but retains the brown color. If the washing is

incomplete, crystals of corrosive sublimate will be unduly conspicuous in the preparation. Camphor may be used instead of iodine to hasten the washing.

The carmines are very brilliant after corrosive sublimate on account of the formation of mercuric carminate, but hæmatoxylin and anilines also give good results. It is claimed, however, that achromatic structures do not stain well. It might be worth while to test something besides the safranin-gentian violet-orange combination before discarding this reagent for cytological work.

Corrosive sublimate material gets very brittle if allowed to remain long in alcohol, and therefore it is better to imbed it as soon as possible.

#### FORMALIN.

Formalin is a comparatively recent addition to the list of killing and fixing agents. It is an excellent preservative, often preserving the color as well as the structure of objects. A 2 or 4 per cent. solution in water is good for filamentous algæ. The material may simply be put into the reagent, and left until needed for use. After a thorough washing in water, any of the usual stains may be used. Some good notes on formalin will be found in the *Botanical Gazette* of March, 1896.

(To be Continued.)

### A Convenient Washing Bottle.

Perhaps the most important step in the preparation of objects for the completed mount is washing out the hardening fluid.

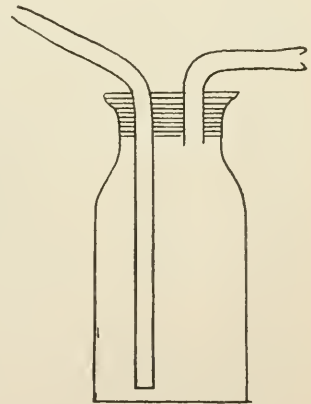
A very simple piece of apparatus seems to accomplish this end most successfully. It consists of a large-mouthed bottle fitted with a two-hole stopper, two short pieces of glass tubing, and, also, a piece of rubber tubing for connections. The stopper should fit tightly, so that the small particles which are being washed cannot become lodged between the stopper and the neck of the bottle. The two pieces of glass tubing are bent and passed through the holes of the stopper, as shown in the cut; the one should reach almost to the bottom of the bottle, while the other should barely pass through the stopper. Before placing the latter tube in position, put a piece of cloth of coarse mesh, or perhaps some wool fibers, over the end, to prevent the particles from being driven out while they are being washed.

The bottle is now ready for use. The objects to be washed, with the hardening fluid, are poured into the bottle, and water is allowed to flow through as long as is necessary. If there is no hydrant at hand, fill a large jar, or bottle, with water, raise it to a higher level than the washing apparatus, connect the two, and allow the water to siphon off through the bottle.

When the washing is completed, the objects can be poured into another vessel, or they can be poured into a cloth, and from there transferred to the first-grade alcohol. Sometimes the washed objects seem very soft; if such is the case, it is better to carry them through the alcohols in the washing bottle.

This is a very simple looking apparatus, but it will be found very convenient, easy to make, and efficient in washing objects of all kinds where the hardening fluid must be removed.

ERNEST I. KIZER.





# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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Mineralogical Literature, by Professors Alfred J. Moses and Lea I. Luquer, Columbia University. In thus enlarging the scope of the JOURNAL it is sought to give comprehensive reviews of the principal papers in all branches of science involving the use of the microscope, thus placing the busy teacher, physician, or student in touch with the most recent literature in all languages.

\* \* \*

SUMMER schools are one of the most important factors in the improvement of science teaching. The establishment and maintenance of suitable summer courses for teachers of all grades has received the support of the most eminent biologists. The work done, and lectures given, very often outclass the best regular college courses. That a large number of science teachers appreciate the opportunities thus offered, and make the most of them, speaks volumes for the science teaching of the future. There are, however, many who do not, as yet, appreciate the advantages now so easily attained. With such schools and laboratories as are each summer opened to teachers at Woods Holl, Cold Spring Harbor, Colorado Springs, Turkey Lake, University of Illinois, and many other places, the teacher is not only able to keep in touch with the most advanced research, but to get in the way of doing a little investigating himself; and no teacher can be a real success, as a teacher, unless he possesses the spirit, at least, of an investigator. The summer school offers another advantage in that, by a natural division of labor and the discussion of the results obtained by each, all acquire a broader view of the field. The meeting together year after year results in the accomplishment of work which would otherwise be an impossibility. When planning the summer vacation, let it include a course at one of the good summer schools and attendance at the meetings of one or more of the scientific societies. This year an especially favorable opportunity is offered, as the American Association for the Advancement of Science, with which are affiliated many other societies devoted to special branches, and the American Microscopical Society hold their meetings at Columbus, Ohio, at nearly the same time.

THE present issue brings the JOURNAL up to date once more, and we have made arrangements whereby no further delays will occur. We desire to thank our readers for the forbearance manifested during the past months, and it is a source of great satisfaction that so very few complaints of delay have reached us, indicating as it does a deep and kindly interest in the JOURNAL. The June number will contain the first of a department of Neurological Literature, to be conducted by Miss Edith M. Brace, University of Rochester, and also the beginning of the department of

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Barnes, C. R.** Plant Life Considered with Especial Reference to Form and Function. New York, Henry Holt & Company. Pp. X+ 428, 415 ff., 1898.

This book, which is intended for use in secondary schools, aims to give an elementary account of the form and functions of plants of all groups. The first part (141 pp.) shows the variety and progressive complexity of the plant body. The second part (65 pp.) deals with physiology and seems rather short in comparison with the chapters on reproduction (97 pp.). The Ecology (61 pp.) follows the texts of Warming and Kerner. Although but few of the numerous excellent illustrations are original, they are nevertheless drawn from more recent sources than those of most botanical texts and will consequently be new to many readers. Teachers will find the laboratory directions very helpful, especially the directions for physiological experiments, which are particularly clear and instructive.

The book is logical in arrangement and literary in style; every live teacher of botany should read it. It is to be feared, however, that the average pupil in the secondary school is not sufficiently advanced to read the more difficult chapters intelligently.

C. J. C.

**Atkinson, G. F.** Elementary Botany. New York, Henry Holt & Company. Pp. XXII+ 444, 509 ff.

This book is also intended for use in the secondary schools. Physiology, which is considered first, occupies 92 pages, morphology 209 pages, and ecology 123 pages. Nearly all of the numerous illustrations are new. The best that can be said of the book is that it presents an account which can be read with interest even by students who have had but little botanical training. The style, however, is often careless and gives one the impression of hasty writing. While both this text and that of Prof. Barnes may be rather difficult for the average pupil of the secondary schools, they nevertheless seem to meet the needs of the schools more nearly than any elementary botany which has preceded them.

C. J. C.

**Guignard, L.** Sur les anthérozoïdes et la double copulation sexuelle chez les végétaux angiospermes. Comptes rendus des séances de l'Acad. d. Sci. 128: 1-8, 1899.

The recent note by Nawaschin<sup>1</sup> on the peculiar behavior of the male cells in *Lilium martagon* has called out a more extended paper by Guignard upon the same subject. The preliminary statements of Nawaschin are confirmed, and the presentation of good figures adds to the conclusiveness of the demonstration.

Of the two male nuclei, one fuses with the female pronucleus, while the other, probably the first one to leave the pollen tube, passes on to fuse with one

(1) Bot. Cent. 77: 62. 1899.

of the polar nuclei. This union is generally with the upper polar, but if the lower one happens to be more conveniently situated, the male nucleus will be attracted to it. Hence the phenomenon cannot be explained by the fact that the two equivalent male nuclei are attracted respectively by the oosphere nucleus and its sister, the upper polar. In either case the ultimate fusion of all three nuclei is recorded.

A case figured, in which the male nucleus, coiled like a closely compressed letter S, lies in the angle between the fusing polars, is cited as presenting a condition which, with the less perfect technique of eight years ago, might have been taken for a pair of fusing centrosomes. The male nucleus in question is figured as lying upon the polars, whereas the most favorable position for finding centrosomes is more to one side. And it will be difficult for many persons to see how such an S-shaped body could be cut so as to show the *two* pairs of fusing centers on opposite sides of the polar nuclei, as they have been figured in the familiar quadrille.

The fusion nucleus, whether of oöspore, or endosperm, preserves the external marks of its dual or triple nature even into prophase stages. Under such conditions as have been described, the increased number of chromosomes in endosperm nuclei may find an easy explanation.

The male nuclei are described as long and variously twisted bodies, many of them presenting forms which, as noted by Nawaschin, suggest a possible motility. While they are devoid of cilia and cytoplasm, they nevertheless are entitled to the name of antherozoids.

It may be well to call attention to somewhat similar observations made by other botanists. Golinski<sup>2</sup> reports male cells in pollen grains and tubes of *Triticum* and other grasses, which resemble the "antherozoides of a fern or of *Chara*." His figures are, unfortunately very unsatisfactory. Mottier<sup>3</sup> figures a coiled male nucleus closely applied to the female, in *Lilium martagon* (see his figure 25). Indications of a male nucleus uniting with the polars have been found in *Lilium philadelphicum* by students at Chicago during this last year. The reviewer has found elongated, and often spirally twisted male cells in the pollen of *Silphium*, the figures for which are soon to be published. It would seem, then, that such reminiscent forms of male cells are to be looked for almost anywhere, since they have been found in such widely separated groups as the Gramineæ and Liliaceæ and the Compositæ.

W. D. MERRELL, Chicago.

**Boirivaut, A.** Recherches sur les Organes de Remplacement chez les Plantes. Ann. d. Sci. Nat. Bot. Ser. 8, 6: 309-400. pl. 17-21. 1898.

The general facts of correlation have been observed and studied for a long time, indeed, many common horticultural practices are based on some knowledge of them. M. A. Boirivaut has recently studied the subject from a new standpoint. He sets out by asking himself the following questions:

Within what limits can new organs replace old ones?

(2) Bot. Cent. 55: 1-17, 65-72, 129-135, 1893.

(3) Jahrb. f. wiss. Bot. 31: 125-158, 1898.

How is the structure of the new organs modified?

To what degree do they acquire the structure of the organs which they replace?

He then gives the result of a number of careful experiments on roots of replacement, showing that the lateral or branch root, which replaces a tap root, not only resembles it in external appearance, but in anatomical structure as well. A second series of experiments brings out the same facts in the case of lateral branches which replace main stems. In another division of his paper he discusses the changes which take place in stems when deprived of leaves at an early stage. Such stems, when compared with normal stems, show considerable modifications of structure, perhaps the most striking of which are the greater development of chlorophyll-bearing tissue and the multiplication of stomata. These latter facts are emphasized by some physiological experiments which show a much greater power for transpiration and fixation of carbon in stems, which have been deprived of leaves, than in normal stems.

A. C. MOORE.

Chicago.

**Goldberger, B. A.** A Ready Supply of *Vaucheria*.  
Bot. Gaz. **27**: May, 1899.

The writer has been able to obtain a good supply of fruiting *Vaucheria* at any time of the year by carefully removing the mats from pots in greenhouses, and throwing them into a jar half full of water. The jar should be placed in good sunlight. In five or six weeks the material may show both methods of reproduction, and has the advantage of being free from dirt and other algæ. The species experimented upon was *Vaucheria sessilis*.

C. J. C.

**Williams, J. L.** New *Fucus* Hybrids. Ann.  
Bot. **13**: 187-188, 1899.

Several years ago Thuret obtained hybrids by fertilizing oöospheres of *Fucus vesiculosus* with antherozoids of *F. serratus*. The present short note records some interesting experiments. Oöospheres of *F. vesiculosus* fertilized by antherozoids of *Ascophyllum* in many cases developed into healthy plants. When antherozoids of *F. serratus* were added to eggs of *Ascophyllum*, about one-fourth the number produced investing walls, and a few segmented but failed to develop further. Antherozoids of *Halidrys* added to eggs of *F. vesiculosus* gave no evidence of fertilization.

C. J. C.

#### RECENT LITERATURE.

**Caldwell, O. W.** The Relation of Bacteria to the Nutrition of Plants. The American Florist. **14**: 1173-1175, 1898.

**Davis, B. M.** Recent Work on the Life-History of the Rhodophyceæ. Bot. Gaz. **27**: 315-320, 1899.

**Giesenhagen, K.** Lehrbuch der Botanik. Second edition. Pp. IX + 406, ff. 528. München & Leipzig. Dr. E. Wolff, Wissenschaftlicher Verlag, 1899.

**Lang, W. H.** On Apogamy and the Development of Sporangia upon Fern Prothallia. Phil. Trans. of the Royal Soc. of London. Series B. **190**: 187-238, pl. 7-11, 1898.

**Mac Dougal, D. T.** Symbiotic Saprophytism. Ann. Bot. **13**: 1-47, pl. 1-2, 1899.

**Murray, G. and Blackman, V. H.** On the nature of the Coccospheres and Habbospheres. Phil. Trans. of the Royal Soc. of London. Ser. B. **190**: 427-441, pl. 15-16, 1898.

**Nemec, B.** Ueber die Karyokinetische Kernteilung in der Wurzelspitze von *Allium cepa*. Jahrb. f. wiss. Bot. **33**: 313-336, pl. 3, 1899.

**Salmon, E. S.** On the Genus *Fissidens*. Ann. Bot. **13**: 103-130, pl. 5-7, 1899.

**Sturch, H. H.** *Harveyella Mirabilis*. Ann. Bot., **13**: 83-102, pl. 3-4, 1899.

**Trow, A. H.** Observations on the Biology and Cytology of a New Variety of *Achlya Americana*. Ann. Bot. **13**: 131-179, pl. 8-10, 1899.



## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
 Agnes M. Claypole, Sage College,  
 Ithaca, N. Y.

## CURRENT LITERATURE.

**Cajal, Ramón y.** Algo sobre la significación fisiológica de la neurógliá. Rev. trim. microgr. t. II, fasc. 1, pp. 33-47, 1897. Abstract in Zeit. f. wiss. Mikros. 15: p. 365.

A technical point is of particular interest in this connection. The author finds that Weigert's neuroglia stain does not differentiate neuroglia fibers alone, but

also more or less non-medullated nerve fibers. Hence, a few axis-cylinders of the stellate cells of the molecular layer of the cerebellum, and some of the basket fibers of the purkinje cells, are brought out. The method, however, gives a complete representation only of the neuroglia. The complex lateral processes of Bergmann's fibers, the delicate branches of the cells of the molecular layer, the thick processes of epithelial cells do not stain at all. The author considers Weigert's method most valuable for the neuroglia of the alba.

A. M. C.

**Apathy, Stefan.** Hæmatein Method. Mittheil. aus der Zool. Stat. zu Neapel. 12: p. 712-718, 1897.

This method is best adapted to invertebrate tissue, and may be used for either fresh or salt water forms. The solution

for staining is made by pouring together equal volumes of (a) one per cent. hæmatein in seventy per cent. alcohol; (b) concentrated glycerine; (c) a mixture of distilled water with one pro mille salicylic acid, three per cent. acetic acid, and nine per cent. alum.

Fixation in sublimate solution (concentrated solution of sublimate in one-half per cent. sodium chloride) is best for demonstrating the nerve cell or the plexus, although Lenker's fluid, picric acid, or other fixing agents may be used. If the material is not to be stained at once, it should be left in ninety per cent. alcohol.

The specimen should not exceed five millimeters in thickness, and should be stained in toto if small enough. Regardless of size, material must be left in the stain at least forty-eight hours, although three days is not too long, and it may be left longer. After staining, wash in distilled or double distilled water, frequently renewed. The duration of the washing is the most difficult point, but once determined, it may always be kept the same. It depends upon the size, the condition of the tissue, and the position of the nervous system.

Neurofibrils are stained dark blue to black, although if left too long everything stains, but the stain may be washed out with distilled water, except from the nucleus. If the water inclines to be acid, wash in slightly alkaline water, then two hours in distilled water. From distilled water, change directly to a quantity of absolute alcohol, and imbed in paraffin, celloidin, or glycerine jelly, and mount in balsam. For paraffin imbedding, chloroform should be used. In celloidin imbedding, avoid much exposure to the light. To imbed in glycerine jelly, place in the jelly immediately after washing in distilled water, keep at an even temperature until all the water is expelled, pour into a mold, place it in absolute alcohol, and cut in absolute alcohol.

E. M. BRACE.

**Busch, Ch. K.** Ueber eine Färbungs methode secundärer Degeneration des Nervensystem mit Osmiumsäure. *Neuro. Centralbl.* 17: 10, p. 476, 1898.

The well known disadvantage of Marchi's method is the poor penetration of osmic acid into the deeper layers of tissue. This can be avoided by mixing

the osmic acid in a solution of sodium iodide. The substance gives osmic acid quicker penetration and fixing powers. A formol hardened preparation was put into a mixture of:

Osmic acid, . . . . .	1.0
Sodium iodide, . . . . .	3.0
Distilled water, . . . . .	300.0

allowed to remain for five to seven days, and passed through the alcohols to celoiden. Sections have the same color as after Marchi's treatment, only the normal tissue is brighter, and consequently the degenerating area stands out more clearly, being visible to the naked eye.

A. M. C.

**Marsson, Dr.** Planktologische Mittheilungen. *Zeits. f. Angew. Mikros.* 4: 169-174, 1898.

The results of five months' observation of the plankton of the Leipsig ponds

are given, with a table of the forms found. There was a pronounced variation in the plant and animal constituents of the plankton, many organisms suddenly appearing and then disappearing after a few days. *Tintinidium fluviatile*, an organism living in a gelatinous tube, made its first appearance in one of the ponds on the twentieth of May; on the twenty-sixth it formed the largest constituent of the plankton. Then it disappeared and did not return. A closely related form, *Codanella lacustris*, appeared in this pond in April, then disappeared entirely and was first found in other ponds in August. Two other ponds, separated only by a road, never contained the same forms. While the one on the south contained quantities of *Volvox aureus*, not a single specimen could be found in the one on the north. In September *Synura uvella* appeared here in large numbers, while there were none at all in the other. Both ponds afforded similar conditions of depth, character of soil, light, and plant growth. Swans frequently went from one to the other, and they were visited by other water birds.

E. M. BRACE.

**Bristol, Dr. L. C.** *Jour. Morph.*, 50: No. 1.

Dr. L. C. Bristol gives a gold chloride method which he has found to give

very delicate results in the study of the nervous system. He recommends it for vertebrate or invertebrate, adult or larval tissue, although there must be special adaptations determined by experiment for each tissue. Formic acid is the variable factor and its action depends upon time and strength. The material is killed in a 10 per cent. or 15 per cent. solution of formic acid, where it is left for five or ten minutes. Without washing, place in a 1 per cent. solution of gold chloride for twenty-five minutes; change, without washing, to a large volume of 10 per cent. formic acid and leave twelve to eighteen hours, or until reduction occurs. Small pieces, not thicker than 5 mm., should be used. Maceration may be avoided by reducing the strength of the acid and the time of action. During reduction the preparation may or may not be left in the sunshine. After reduction pass the tissues through the alcohols to chloroform and imbed as soon as possible. Sections should be about 18  $\mu$  thick.

E. M. BRACE.

**A New Microscope Objective for Zoological and Other Biological Investigations under Water.**  
Zeits. f. Wiss. Zööl. u. f. Mikr. Tech., 12:  
pp. 1-2, 1898.

Dr. H. Harting calls attention to a microscope objective manufactured by Carl Zeiss, which is suited to the observation of living organisms under water, in cases where a wide field and depth of view are desired more than a high degree of magnification.

It is called the "Planktonsucher." It has a focus of 33 mm., a working distance of 36 mm., and a numerical aperture of 0.11.

For a tube-length of 160 mm. and using the Huyghenian eye-pieces one to five, by Zeiss, the following values may be obtained for magnification and visual field:

Huyghenian eye-piece.	Field. mm.	Magnification.
1	3.5	25
2	3.3	35
2*	4.2	35
3	2.4	50
4	2.0	60
5	1.7	80

Eye-piece 2\* differs from eye-piece 2 only by its larger visual field. With eye-piece 5 the plankton lens gives perfectly clear images so that a magnification of eighty may be conveniently used. Even with the wide field of eye-piece 2 the image is perfectly clear and free from astigmatism.

For their use in narrow water cells the lenses are fastened in the end of cylindrical nicked tubes, so that the water cannot penetrate.

For holding small organisms in the bottom of the cell, three small strips of glass may be fastened to the bottom, from 0.5 to 1 mm. high, and a strong cover-glass, with diameter a little less than that of the cell, placed on them, so that the object may be kept in a thin layer of uniform depth.

E. M. BRACE.

**Cleghorn, Allen.** Sympathetic Ganglia and Blood Pressure. Jour. Boston Soc. Med. Sc., 3: 207-208, 1899.

It was found that an extract of the sympathetic ganglia of the cat or dog produced a fall in blood-pressure when injected into the jugular or femoral vein. This property of lowering the blood-pressure belongs to the superior and inferior cervical, stellate, and the large ganglia of the solar plexus. Control experiments with spinal ganglia, spinal cord, brain matter, nerve and abdominal tissue produced no results. The sympathetic extract was prepared by macerating the ganglia in glycerine for twenty-four hours, then enough saline solution (0.8 per cent.) was added to slightly thin the extract, and the whole filtered under pressure.

E. M. BRACE.

**Melissenos, C.** Ueber Erythroblasten des grossen Netzes. Anat. Anz. 15: No. 22, pp. 430-435.

As is well known at the present time, red blood corpuscles are found during embryonic life in the liver, the spleen, and red bone marrow. In later life the marrow alone gives origin to large nucleated red cells, that by the loss of their nuclei become ordinary red corpuscles. This investigation was conducted on embryo, and newly born cats at a time when the usual blood-forming organs showed activity, the greater omentum being the region particularly investigated.

The method of investigation was to inject into the peritoneum of the cats of various embryonic ages, Altmann's liquid or sublimate. After half an hour the spleen, stomach, and greater omentum were removed and put for an hour in the same kind of fluid that was used for injection. Thorough washing in distilled water was followed by staining in hæmatoxylin for a few minutes; after more washing it was allowed to remain in eosin for several minutes, and after washing was brought on to a slide, stretched, and mounted in balsam, clearing in bergamot oil.

The author finds, in that part which lies between the curvature of the stomach and the spleen, groups of erythroblasts similar to those found in other organs. These erythroblasts become transformed into red blood corpuscles by the extrusion of their nuclei. There are found among these cells, as in all centers of similar growth, the characteristic "Riesenzellen"; thus this author adds evidence to the extrusion theory, in the disputed question concerning the fate of the nucleus of the red blood cell.

A. M. C.

**Levy, A. G.** The Changes Occurring in the Blood of Dogs after the Removal of the Thyroid. *Jour. Path. and Bact.*, **5**: No. 3, pp. 316-330.

These experiments were performed with reference to microscopic and chemical changes in the blood after the complete removal of thyroids and

parathyroids in dogs. In every case careful examinations were made of the blood before operating, and time allowed for a complete recovery from the bleeding necessary for the process. Estimates were made of the amount of hæmoglobin present, the number of red and white corpuscles, the specific gravity, the fibrin, proteids, total solids, ash, and total nitrogen. Experiments were carried on with twelve animals of various kinds and conditions. The author finds several interesting results. It appears that the reduction in number of red and white blood corpuscles is not a constant feature, and when anæmia does result its degree is very uncertain.

A. M. C.

**Morgan, T. H.** A Confirmation of Spallanzani's Discovery of an Earthworm Regenerating a Tail in Place of a Head. *Anat. Anz.* **15**: No. 21, pp. 407-410; 9 figs.

As long ago as 1768 Spallanzani states that he found a species of earthworm in which a tail was regenerated instead of a head. Until now no cor-

roboration of this remarkable fact has been found, but Morgan describes in the above article a clearly similar case. The experiments were carried on for five months, and a number of worms cut in two at different distances from the anterior end. Several of these regenerated a smaller part in every way similar to a tail and showing a larger number of segments than the normal regenerated head. Histological examination showed that the ventral nerve cord extended to the end of the piece, and there was no evidence of a dorsal brain. Not only was this evidence of its nature clear, but in the new segments, the nephridial tubes opened in an opposite direction from those of the other part, and hence proved the fact that the regenerated piece was a tail, and not as hitherto supposed by the present author, as well as by earlier workers in such cases, an undeveloped head end.

A. M. C.



## RECENT LITERATURE.

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- Swanzoff, N.** Ueber die physiologische Bedeutung des processes der Eireifung, 1 Taf., Bull. de la Soc. Impér. des Naturalistes de Moscou, Année, 1897, N. S. T. 11, S., 355-367 (Befruchtung unreifer Eier v. Echinodermen), 1898.
- Kohn, A.** Die chromaffinen Zellen des Sympathicus. Anat. Anz. 15: No. 21, pp. 393-400, 1899.
- Montgomery, Thos. H.** Chromatin Reduction in the Hemiptera; a Correction. Zool. Anz 22: No. 580, pp. 76-77.
- Marinesco, G.** Recherches sur la biologie de la cellule nerveuse, Arch., f. Anat. u. Physiol., Physiol. Abth., pp. 89-111, 1899.
- Pappenheim, A.** Der Lehre von der Kern-Ausstossung der rothen Blutzellen in ihrer Vertretung durch C. S. Engel., Arch. f. pathol. Anat. u. Physiol. 155: No. 1, pp. 123-134.
- Ascoli, Maurizio.** Ueber die Blutbildung bei der Pricke. Arch. f. Mikros. Anat. u. Entwicklungsgesch. 53: H. 4, pp. 623-631.
- Hesse, R.** Untersuchungen ueber die Organe der Lichtempfindung bei niederen Thieren: Die Augen der Polychaeten Anneliden. 5 Taf. Zeitschr. f. wiss. Zool. 65: pp. 446-516.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review  
to H. H. Waite, 710 East Catherine street,  
Ann Arbor, Michigan.

- Daubler, C.** Ueber die baktericide Kraft der Leukocytenstoffe verschiedener Thierspecies und ihr Verhältniss zu den baktericiden Stoffen des Blutserus. Centrblt. f. Bakt. 25: 129-141 and 181-187, 1899.
- In order to ascertain the bactericidal power of blood-serum in comparison with that of the products of the leucocytes from pleural exudates and pus, the author of this paper has used as a means of determination these products obtained from the dog, rabbit, and guinea pig. The most important results obtained from these investigations may be summed up as follows:
1. The bactericidal power of the leucocytes of different species of animals, as the dog, rabbit, and guinea pig, is in itself variable, and also varies in action on certain pathogenic bacteria.
  2. In comparing the bactericidal power of sterile pus, and of pleural exudate from the same animal, the pus is shown to have absolutely higher but relatively less bactericidal power than the pleural exudate, and does not lose its effectiveness for some days.
  3. The isolated living as well as dead leucocytes of the dog, which possess a stronger bactericidal power than the plasma, maintain this property after heating at 60 degrees C., with the separation of a neutral sterile fluid which does not produce a pronounced agglutination.
  4. The vital processes in the animal body aid the bactericidal effectiveness of the leucocytes of animals of other species, shown to be active *in vitro*, after the introduction of the latter into an animal of another species. The foreign leucocytes become disintegrated; those constituting an integral part of the animal do not diminish in power or vital activity.
  5. During the process which renders the dog immune to typhoid fever, no bactericidal products are deposited in the leucocytes. Their bactericidal power remains unchanged.

6. The bactericidal products of blood serum and of leucocytes cannot be identical.

H. H. W.

**Smith, Theobald.** One of the Conditions under which Discontinuous Sterilization may be Ineffective. *Jour. Exp. Med.* 3: 647-650, 1898.

It was found that shallow layers of bouillon, steamed in the Arnold sterilizer for an hour, on three or four successive days, developed anaërobes

after some days' growth of diphtheria bacilli which had formed a membrane over the surface and excluded the oxygen. In other cases, aërobes on solid culture media produced changes, that aroused growth in dormant spores of anaërobes, which apparently do not find conditions favorable for development in the intervals between the heating of the culture medium. To insure perfect sterilization, the culture medium may be heated to a temperature of 110° to 115° C. Where apparatus for heating to such a temperature is not available, the anaërobes may be destroyed by steaming the bouillon three or four times in round liter flasks filled to the neck, and afterward placing the flasks in the incubator, where the anaërobes will develop in a couple of days. The bouillon may then be poured or siphoned into sterile culture flasks if it is to be used in shallow layers.

E. M. BRACE.

**Hektoen, Ludvig.** The Fate of the Giant Cells which Form in the Absorption of Coagulated Blood Serum in the Anterior Chamber of the Rabbit's Eye. *Jour. Exp. Med.* 3: 573-578, 1898.

Bits of sterile solidified blood serum were inserted into the anterior chamber of the rabbit's eye, and animals were killed at intervals of from a few days

to six weeks after the operation. During the early stages there is a limited emigration of polymorphonuclear leucocytes, some of which returned to the iris after becoming loaded with granules of serum. New cells are formed which accumulate around the foreign body, and most of them take up granules from it with their cytoplasm. During this time there are formed multinucleated giant cells or plasmodial masses, which appear to result from the fusion of many small cells. At first these giant cells are more or less circular, with smooth margins. Later, they become oblong in shape and free from granules, but there is no degeneration nor any immigration of small cells or leucocytes into the giant cells. In one instance a dividing nucleus was found in a giant cell.

As the serum disappears the giant cells become marked with lines of cleavage which map out spindle-shaped cells. These uninuclear masses, partly detached, as well as small cells resembling them, are found about the giant cells.

The writer concludes that giant cells formed under these conditions resolve themselves into small cells which unite with other newly formed cells to form a fibrillated tissue resembling the cornea in structure. He also considers that the results furnish evidence opposed to the general teaching that giant cells of tuberculosis are necrobiotic from the time of their formation.

H. H. W.

**Frankland, Percy, Ph. D., F. R. S.** The Action of Bacteria on the Photographic Plate. *Centralbl. f. Bakt.* 24: 609-612, 1898.

Photographic plates were placed about half an inch above and below gelatin cultures of *Proteus vulgaris*, and

*Bacillus coli communis*, to determine what effects might be produced. Both cultures gave strong light effects on the sensitive plates over the dishes, but the

influence did not penetrate the glass beneath, and the plate under the dish was not affected. Streak cultures gave a stronger picture, and sensitive films, dropped into the dishes in direct contact with the growths, gave a distinct image of the colonies. Phosphorescent forms act much more powerfully, and their influence will also traverse glass. The sensitive films are affected by both liquefying and non-liquefying forms, probably through the gases which are evolved and which react upon the film.

E. M. BRACE.

**Kern.** Ueber die Kapsel des Anthrax Bacillus. Centralbl. f. Bakt. Abstr. 1 Bd. 22: No. 6 u. 7.

The writer demonstrated the presence of a capsule for anthrax bacilli from agar, bouillon, gelatin, serum, and potato

cultures. Each bacillus has its own capsule, but it is more easily seen in bacilli that are at least two days old. In young bacilli it is very small, larger in older forms, and sometimes swollen out like a vesicle. The technique consisted in pouring anilin water fuchsin, or gentian violet solution, Ziehl's carbolic fuchsin or Löffler's methylen-blue, over a cover-glass preparation. This was heated until it steamed, and the heating repeated five or six times, pausing a moment between the heatings. The preparation was then rinsed and examined in water.

E. M. BRACE.

**Dawson, Charles F., M. D.** A Hermetic Seal for Bacterial Cultures. Nat. Med. Rev. 8: 264, 1898.

Two methods are given for sealing bacterial cultures. In one, the plugged end of the tube is flamed and covered

with a flamed round cover slip. Over this is stretched a piece of sheet gelatin which has been soaked a moment in a 1/1000 solution of bichloride of mercury and kneaded until sticky. Press the gelatin mantle against the tube with a small rubber band, and after two or three minutes run a knife around the band and remove any superfluous gelatin. After pressing the cap into place, cover with a coating of varnish compounded as follows: absolute alcohol, 100 parts; white shellac, 45 parts; balsam copaiba, 4 parts. In the other method, the tube is plugged and flamed and the cotton depressed about one-fourth of an inch, and the space above filled with a fluid mixture of plaster of paris and eosrosine sublimate or other aqueous solution of a germicide. When this has dried cover the surface with hot paraffin. Both seals may be removed without destroying the tube.

E. M. BRACE.

**Malarial Studies in Italy.** Philadel. Med. Jour. 111, 1898.

Italian observers have been paying considerable attention to the etiology and

therapeutics of malaria. Dr. Amico Bingnami has decided that malaria is "a disease of inoculation" and has experimented on the possibility of engendering malarial fever by means of the punctures of mosquitoes. Dr. Grassi, carrying the experiments further, finds that the insects which are probably the purveyors of malaria are *Anopheles claviger* (Fabr.), *Culex penicillaris* (Rondani), and *Culex hortensis* (Ficalbi.)

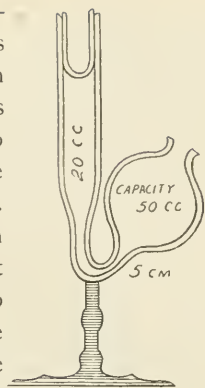
In addition to this, a malarial infection has been found in bats which is due to parasites resembling those of human malaria. The amebæ are present in great numbers. The annular forms are those chiefly seen, but some pigmented forms are also present.

E. M. BRACE.

**Hill, Hibbert Winslow.** A Modification of the Fermentation Tube for Bacteriological Work. Jour. Boston Soc. Med. Sc. **3**: 137-138, 1899.

much liquid as the closed branch. This is designed to prevent wetting of the cotton plugs in sterilization. The opening into the bulb is made large enough to admit a 10 cc. pipette. The closed upper end of the ordinary fermentation tube is replaced by a thimble-shaped stopper, ground to fit snugly. To examine the liquid in the closed branch when no gas has been formed, the cotton plug in the bulb may be replaced by a closely fitting sterile rubber stopper. In the case of a gas-forming bacillus a similar method is followed.

E. M. BRACE.



Fermentation tube, with movable top for closed branch.

**Bissell, William, G., M. D.** Second-hand Clothing.

Investigation has shown that these men deal largely in clothing that was formerly worn by deceased persons, and as it is estimated that more than one-half of the human race dies from contagious diseases, the danger of infection from this clothing becomes apparent. The custom in organizations uniformed with state property, of reissuing the uniform and equipment of a discharged member to an incoming recruit, is also shown to be unsafe. Pockets were removed from uniforms of men with pulmonary tuberculosis who were leaving their regiment; the pockets were soaked in distilled water, the product was centrifugated several times, and guinea pigs injected with the fluid. Several of the animals died, and examination showed that tuberculosis had developed. The writer recommends that the matter be considered by health authorities and legislative bodies.

Attention is called to the danger of infection from clothing sold by second-

E. M. BRACE.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Pathological Laboratory, Boston City Hospital, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

### CURRENT LITERATURE.

**Feldbauseh.** Der Einfluss verschiedener Stoffe auf die rothen Blutkörperchen und Bedeutung der letzteren für Gerinnung. Virchow's Archiv. **155**: 1899.

the blood platelets are merely broken-down red blood corpuscles.

Feldbauseh concurs in the opinion of Mosso, Wassow, and Arnold that red blood corpuscles play the chief part in the coagulation of the blood, and that

**Goodale, J. H.** A Contribution to the Pathological Histology of Acute Tonsillitis. Jour. Boston Soc. Med. Sc., **3**: No. 4.

the presence of one or more varieties of the pyogenic cocci. In all cases he

In this report, Goodale gives the results of a study of sixteen cases of acute tonsillitis. In all cases cultures showed



found a diffuse proliferation of lymphoid cells and of the endothelial cells of the reticulum. Some of the latter showed phagocytic properties, as they contained lymphoid cells, red blood corpuscles, and nuclear fragments. In the loose mucous membrane of the tonsil the plasma cells of Unna were present in larger numbers than normal. In four cases small abscesses in the interior of the follicles were found. The growth of these abscesses was always in the direction of the nearest crypt. Phagocytic cells were particularly numerous in the periphery of these areas. The article is illustrated by six photomicrographs.

R. M. P.

**Arnold, J.** Zur Morphologie Intravasculären Gerinnung und Pfropfbildung. Virchow's Archiv. 155: Heft 1, 1899.

J. Arnold, as the result of his observations, believes that the blood platelets are formed from red blood corpuscles,

and that fibrin formation and thrombosis are closely related to degenerative changes in the red blood corpuscles. A simple diminution of size, leading to the formation of microcytes, or to complete disappearance of the corpuscle, he terms Erythrocytolysis. The extrusion of round refractive granules ("dust bodies" of Müller), and the separation at the periphery of small particles, he terms Erythrocytorrhexis. Erythrocytoschisis is the term used to describe the breaking up of red blood corpuscles into disc-like bodies. In the mesenteric vessels of living animals he observed the formation of blood platelets from red blood corpuscles by the last two forms of degeneration. This observation he believes settles the question of their origin. In thrombosis, in rabbits, produced by the inoculation of foreign bodies, and in both vital and agonal thrombi in man, he observed in red blood corpuscles all three of these degenerative changes.

R. M. P.

**Councilman, W. T.** The Character of the Cellular Exudate in Acute Keratitis of the Rabbit. Jour. Boston Soc.-Med. Sc., 3: No. 5.

In a very extensive piece of work Councilman makes a study of the following points: (1) the character of the leucocytes which emigrate; (2) from

what vessels do they come? (3) the changes which they undergo in the course of their migration; (4) the changes which take place in the corneal corpuscles; (5) under what conditions, and how does the formation of new blood vessels take place? The principal irritant used was a pure culture of the staphylococcus pyogenes aureus, introduced by scratching the center of the cornea with a knife. Sections were examined from animals killed after 6, 8, 12, 18, 24, 36, and 48 hours, and daily up to the thirteenth day. Nitrate of silver, caustic potash, and chloride of zinc were also used as irritants.

Results: (1 and 3) Three forms of leucocytes were found: first, a leucocyte with fragmented nucleus, a limiting membrane, and a granular protoplasm staining intensely with eosin; this cell corresponds to the polymorphonuclear leucocyte of human blood. Second, a slightly granular leucocyte, with a horse-shoe nucleus and no limiting membrane. Third, a lymphocyte with reticular nucleus and a very narrow rim of protoplasm. The granular leucocytes appear early in the inflammatory process and are the most numerous. They travel between the corneal fibers, and are often so drawn out as to stretch entirely across the field of

an oil-immersion lens. Often they leave behind them small particles of their granular protoplasm. Leucocytes of the second form generally appear eighteen to twenty-four hours after inoculation. The lymphocytes appear about the fourth day. About the fifth day or later, in the outer third of the cornea, plasma cells are seen. These cells are formed from lymphoid cells by the gradual formation of protoplasm around the nucleus, and are amœboid. The transformation from lymphoid cells to plasma cells probably takes place outside the cornea.

(2 and 5) In a flat section of the cornea, including the sclera and conjunctiva, a plexus of veins can be seen just outside the cornea. From these veins the emigration of cells takes place and the new blood vessels are formed. The first step in the formation of the new blood vessels is seen forty-eight hours after the injury, in the proliferation of the cells of the large veins and the resulting formation of protoplasmic outgrowths. Red blood corpuscles afterwards appear between and around the cells forming these processes. In other cases red blood corpuscles appear in the cell spaces immediately around blood vessels. They seem to become surrounded by the growing cells, and in this way are converted into vessels.

(4) Changes in the corneal corpuscles are both regressive and progressive. The cells directly acted on by the irritant are entirely destroyed. In the immediate periphery of the eschar, cells are seen whose nuclei have separated into two, and sometimes three or four fragments; the protoplasm also is fragmented. This degenerative change corresponds to the so-called direct nuclear division. In cases where a caustic was used as the irritant, the formation of new corneal corpuscles is indicated by the presence of many mitotic figures. The growing cells often have cell inclusions, consisting of fragments of necrotic corneal corpuscles. The corneal corpuscles take no part in the formation of new blood vessels.

The article is illustrated by twenty-two excellent photomicrographs.

R. M. P.

## NEWS AND NOTES.

PICRO-CARMINE AND ALUM-CARMINE AS COUNTER-STAINS.—I notice in the October issue of the JOURNAL an article headed "Picro-Carmine and Alum-Carmine as Counter-stains," in which the author states that he has never been able to obtain good results with picro-carmine as a counter-stain for bacteria in tissues.

As I have had some experience in such staining, I venture to offer some suggestions, some of which may be more or less well known, and all of which I can recommend from personal use as being reliable.

If sections be first stained with logwood picro-carmine, Gram's method of bacterial staining may subsequently be used with very satisfactory results.

As a counter stain alum-carmine alone gives only a nuclear stain and leaves the cytoplasm practically untouched. I have found that better results can be obtained by first staining in alum-carmine or borax-carmine, then carrying the section through the regular Gram's process, and lastly leaving the section for half a minute in the following solution, then alcohol, creasote, and balsam :

Sodium sulph-indigotate	-	-	-	-	0.1 gram.
Carbolic acid, 5 per cent. Aq. Sol.	-	-	-	-	100 cc.

This stains the nuclei red, the cell bodies apple-green, and the bacteria purple (if gentian violet was used in the Gram's solution).

Lithium-carmin-Gram's method:

Lithium-carmin, 10 minutes.

Acid alcohol (1 per cent. HCl in 70 per cent. alcohol), 1 minute.

Pure alcohol, 95 per cent., 3 minutes.

Proceed with Gram's staining as usual.

Results—nuclei red, cytoplasm pink, bacteria purple.

Picro-lithium carmin may be used in the same manner as given above for picro-carmin.

The following may be used after completion of decoloration in Gram's method:

Bismarck brown, 2 per cent. aq. sol., 3 minutes.

Eosin in 70 per cent. alcohol, 1 minute.

Logwood (Delafield's), 3 minutes.

Then in acid fuchsin 1 per cent., orange G Sat. Aq. Sol. aa., 2 minutes.

Logwood (over-stain), 10 minutes.

Picric acid Sat. Aq. Sol., 100 cc., 5 minutes.

I have lately used the following stain, which, so far as I know, is original with me, with very pleasing results, both for celloidin and paraffin sections. The only disadvantage which I can find is in the slightly increased length of time required.

Stain for 15 minutes in logwood, 12 drops; sulph-indigotate of soda sol., 2 drops. Decolorize in nitric acid, 1 per cent., 20 drops. Dehydrate, fix, and darken the stain in alcohol, 95 per cent., 20 drops.

Next proceed as in the ordinary Gram's staining, but substitute basic fuchsin for the gentian violet usually employed:

Anilin water fuchsin, 10 minutes.

Gram's sol. of iodine, 2 minutes.

Alcohol, 95 per cent.,  $\frac{1}{2}$  minute.

Acid alcohol, 10 seconds.

Alcohol, 95 per cent., 5 minutes.

Creasote (best beechwood), 3 minutes.

Mount in canada balsam.

Results—Nuclei deep blue, cytoplasm apple green, bacteria bright cherry red. This method brings out structural detail in the tissue better than any of the bacterial counter-stains which it has been my good fortune to have opportunity to use.

LOUIS LEROY, M. D.

Vanderbilt University Medical Dept.

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ABSTRACT OF PAPER BY DR. B. MEADE BOLTON, ENTITLED, "THE SIGNIFICANCE OF THE RESULTS OF BACTERIOLOGICAL EXAMINATION FOR THE DIPHTHERIA AND TUBERCLE BACILLI."—After a brief description of bacteria in general, as to size, shape, method of reproduction, etc., the paper was devoted to the diseases diphtheria and tuberculosis. Statistics show that in Paris, during

the six years from 1886 to 1892, the number of deaths due to diphtheria was 10,913. The chief cities of Germany present similar statistics. One-tenth of all persons who die, die of tuberculosis. During recent years the mortality from both these diseases has been greatly reduced, and they may now be recognized in their initial stages, before serious symptoms have developed. The earlier the treatment, the better the chances for recovery. In case of diphtheria, antitoxin is now quite generally administered when the disease is first suspected (preparation of antitoxin explained). The diphtheria bacillus is probably conveyed by little particles of membrane expelled by coughing and sneezing. It will grow under somewhat less favorable conditions than the tubercle bacillus, but is probably an obligatory parasite. The bacilli of neither of the two diseases considered are found in the breath. Flügge claims to have demonstrated that the danger from proximity to tuberculous persons lies chiefly in breathing the minute air bubbles coughed up by the patient, and to a much less extent from the dried sputa. Theobald Smith claims to have demonstrated that human tuberculosis and that of cattle are not identical, although their respective bacilli bear a close resemblance to each other. This authority holds that the chief danger from tuberculosis lies in the intimate association with tuberculous persons and that the danger from dairy products is not well founded. The tubercle germ, like that of diphtheria, is probably an obligatory parasite. As yet, no specific is known for the treatment of tuberculosis. In Koch's treatment it is sought to produce such a condition in the body of the patient that the bacilli will be unable to find suitable conditions for their growth. Drawings were presented of the diphtheria and tubercle bacilli showing the different forms which they may assume.—*Read before the New Jersey State Microscopical Society, Feb. 27, 1899.*

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PHOTOGRAPHIC AND MICROSCOPICAL SECTION OF FRANKLIN INSTITUTE.—At a recent meeting of the chemical section of Franklin Institute, plans were made for the organization of a photographic and microscopical branch, the meetings to be held the first Tuesday of each month. Lantern pictures in natural colors were exhibited, H. F. J. Porter of Bethlehem showing pictures taken by the McDonough method. The pictures were taken through mica screens ruled 300 lines to the inch, the lines being colored alternately red, green, and blue, the combination of these colors producing all the other colors. Fred E. Ives showed pictures by the methods of his own invention of taking three pictures simultaneously side by side on one plate. The three are projected through red, green, and purple screens so that the images produce one picture in natural colors upon the canvas. This method gives very smooth and brilliant results.

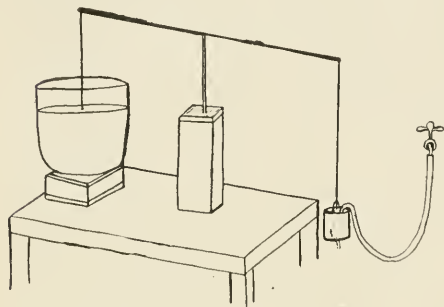
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ON KEEPING MEDUSÆ AND OTHER FREE-SWIMMING MARINE ANIMALS ALIVE IN SMALL AQUARIA.—A method of overcoming the difficulties frequently found in keeping free-swimming invertebrates and pelagic larvæ in aquaria, has been used in Plymouth Laboratory, England. It was noticed that medusæ in the seas simply float along with the tide, without often pulsating the umbrella, and this was taken as a suggestion that slight currents in the water



which would keep the medusæ floating, independently of their pulsations, would produce good results.

Such currents may be made by means of a glass plate suspended in the water. This plate has a glass rod passing through a hole in its center, while the other end of the rod is attached to a light wooden beam which works on a hinge at the center, and has a small bucket, fitted with a self-emptying siphon, and supplied with water by means of a rubber tube attached to the fresh-water supply on the opposite end.



The weights of the bucket and glass plate are arranged so that the plate moves up and down in the sea water as the bucket alternately fills and empties. The weights may be delicately adjusted by means of shot placed in a suitable holder at the end of the beam, and in this way the

motion may be made as slow as desired. The length of the stroke is regulated by two stops, and a slit in the cover of the jar, through which the rod passes, prevents the plate from striking the sides of the jar.

If desired, several plungers may be worked in the same way. The water may be aerated, by putting a funnel with a small hole in the top, in place of the glass plate. The apparatus is arranged so that the funnel comes out of the water with the upward stroke of the plunger, and with the downward stroke, returns full of air, which bubbles up through the water.

Copepods, crustacean, annelidan, and molluscan larvæ were put in the jars as food for the medusæ, and many of the larvæ which were not eaten developed to the adult form.—*Nature*.

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CEMENT FOR FLUID MOUNTS.—The note on cements for fluid mounts in the March JOURNAL leads me to speak of the German "Maskenlack." I first used it in the laboratory of Professor Kny in 1888, and glycerine mounts made then are in good condition to-day. It is an alcoholic cement, but perfectly satisfactory for any non-alcoholic preservatives that we have used.

Mt. Holyoke College.

HENRIETTA E. HOOKER.

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NEW BIOLOGICAL STATION.—A new station will be open at Flathead Lake, Montana, during July and August. The station will be equipped for making collections of either aquatic or land forms of life. There will be a laboratory and facilities for photography. In addition to the advantages for study and for comparison of methods in work, there will be opportunities for investigators to study special groups and to make collections in remote regions. Information regarding the station will be given by Professor M. J. Elrod, University of Montana, Missoula, Mont.

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## Methods of Studying and Mounting Protozoa.

Among the various manuals devoted to microscopical methods—collecting, killing, staining, etc., few accessible to the amateur give any particular attention to the technique of preparing the Protozoa for study, even in the living state, and more particularly for making the permanent mounting of such for critical study or preservation. Lee, in the *Vade-Mecum*, gives a few paragraphs on the subject, and cites a few notes from a variety of sources, few of which are directly available to the beginner.

In view of the interest, and importance as well, it has occurred to me that a brief summary of methods, and some account of personal experience, might prove helpful to readers of the JOURNAL, and thus in a way contribute to continued interest in the microscope, both as a means of entertainment and as a source of important information in the lines of applied microscopy, as well as to teachers whose work lies along the lines indicated. A similar contribution several years ago to the *American Monthly Microscopical Journal*, Vol. X, elicited a number of inquiries which implied a wider interest in the general subject than would at first thought seem likely. To mention only teachers of zoölogy, who know all too well how uncertain is a supply of such organisms in the living state for general class-work at a given time, and who would gladly welcome some means by which even the less satisfactory mounted specimen could be readily available in an emergency, is, I take it, sufficient warrant for the time and space necessary for a statement of "*how it may be done.*"

As preliminary, it may not be amiss to briefly consider methods for the critical study of living specimens. In the case of *Amœba*, or the more or less sedentary Protozoa, little precaution in the way of supporting the cover-glass, or rendering the specimens quiescent, is necessary. But for *Paramœcium*, *Stylonichia*, and others of similar activity, some method of restricting their movements is a practical necessity. The use of certain narcotics, such as chloral hydrate, cocaine, nicotine, etc., I have not found satisfactory, as sooner or later its presence sets up pathological conditions which materially vitiate the very ends sought in such study. The same is somewhat true of the use of such gelatinous substances as cherry-gum, etc., for checking their activities. In my own experience nothing has proved more satisfactory than a few shreds of absorbent cotton, or a fine alga, under the cover-glass, so tangled as to limit the movements within a narrow

limit. This in nowise interferes with the normal activities of the organism, yet effectively restricts the locomotory actions almost to any degree desired, and allows, in most cases, the vital activities quite easy of observation. It has the further advantage that fresh water may be added at any time without special danger of losing the specimen.

Intra vitam staining may be satisfactorily done with many of the Protozoa, and with a variety of stains, among which methylen blue, methylen green, methyl violet, in very dilute solutions, give good results. Congo red has been recommended, and is said to not affect the specimen deleteriously even when used in strong watery solutions. Of course, in this method of staining the object to be attained is the differential condition of the organism in life, and therefore it must be kept under fairly constant observation in order to distinguish the varying effects at varying states of activity, as they approach their maximum or decline. But at no time must there be expected the clearness and sharpness of nuclear definition, etc., as may be secured with the use of one of the specific nuclear stains applied in the usual way.

*Killing, Fixing, etc.*—In keeping with the last suggestion, that the best results can only be realized by the application of the more usual histological, or rather cytological methods, attention will most naturally be directed along that line. And, as in almost any similar research, so here, the first requisite will be material in great abundance. For, as will be seen later, the very operations will necessarily involve the waste or loss of considerable numbers of specimens. It may, however, be noted in this connection that even isolated specimens may be killed, stained, and finally mounted directly upon the slide. But since, in any case, one of the fundamental factors consists in the killing and fixing of the organism as nearly instantaneously as practicable in order that the least possible distortion may result, this may as well be considered in this connection. For this purpose recourse must usually be had to an active poison, which while killing will leave the cell as little altered in form and character as possible. To do this by introducing the reagent under the cover-glass and bringing it quickly into contact with all portions of the organism is much more difficult than by the process of plunging a whole mass of specimens at once into a quantity of the reagent. However, it may be done, of course. In my own experience one of the best reagents for this is Lang's fluid, hot, and applied to the edge of the cover and drawn under with blotting paper applied to the opposite edge. This must be followed by a removal of the excess of the reagent by washing it out with water applied in a similar way. And then, furthermore, the application of the appropriate stain must be similarly made, its excess removed, dehydration by the use of alcohols of increasing strength, clearing, and finally mounting in the desired medium, usually balsam. As will be seen, such a method must be at best a somewhat difficult one, and, except under special necessity, hardly worth the time and trouble involved.

The method sometimes advocated of killing specimens on the slide or cover, after the method commonly suggested for the fixing of bacteria or blood corpuscles, namely, of killing over the flame of a lamp or burner, and drying by desiccation, I have found absolutely worthless for any of the more complex Pro-

tozoa, or even for *Amœba*, since they invariably suffer such distortion as to be rendered practicably indistinguishable.

It remains, therefore, to next direct attention to what seems to me to be the only generally practicable method. It has before been pointed out that material must be at hand in considerable quantities, since in almost every case a very considerable amount will be lost in the operation, or destroyed. If the material be *Paramœcium*, let specimens be taken from the source of supply, aquarium, hay-infusion, etc., with a pipette, and put into a large watch-glass or similar receptacle. Then with a finer pipette draw off the surplus of water from the surface as far as may be done without removing too many of the specimens, an operation which may be facilitated by the aid of a lens or dissecting microscope. If necessary this may be further reduced by the use of a thread siphon, which by its capillarity will withdraw even the last drop, a matter to be guarded against by observing the process from time to time. The same process may be employed for any of the ordinary infusoria, or slightly modified, as circumstances may suggest.

With this part of the process realized, that of killing may be for the most part done successfully with any of several media. It may be well to epitomize several methods, suggested by various workers.

Cattaneo uses a watery solution of chloride of palladium, and claims for it good results. He also suggests chromic, picric, and picro-sulphuric acids, and bichromate of potash. For staining he used carmine or picro-carmine.

Brass employs the following formula for killing: chromic acid, 1 part; acetic acid, 1 part; water, 400 to 1000 parts. For opaque protozoa, he kills in picro-sulphuric acid, then removes to boiling water for a few minutes, then into very dilute ammonia, in which they resume the normal form and size. The ammonia is then neutralized by dilute acid, the preparation stained with borax carmine, washed, and mounted in dilute glycerine.

Certes fixes with two per cent osmic acid, or with the vapor of the acid, from ten to thirty minutes; then stains with borax carmine or eosin, and mounts in glycerine.

Kent and Berthold use potassium iodide, which is said to act very much as osmic acid, and without the deep blackening incident to the latter. They also recommend the vapor of iodine for killing.

Fol has found that in cases where such media as osmic acid, picro-sulphuric acid, etc., have failed, that per-chloride of iron gives good results. By washing in alcohol, staining in gallic acid, the nuclei were well developed, and the preparations were permanently mounted in glycerine or balsam.

In my own experience I have found a saturated solution of bichloride of mercury, to which has been added one per cent. acetic acid, a most excellent medium for killing. In certain cases a hot solution has been found useful, as being more rapid in its results, and producing little or no deformity of the cells. Other media giving good results are Perenyi's fluid, picro-sulphuric acid, osmic acid, one per cent., or in the form of Flemming's fluid.

In general, any reagent which acts rapidly and without undue distortion to the organism may be employed, and much has been said concerning the use of



such media as formalin, but in my own experience the latter has not proven satisfactory, and in certain exceptional cases I have not as yet found anything adequate. For example, the Vorticellidæ are, so far as my own experience goes, not easily killed in an expanded condition by any medium known to me. In such cases resort must be had to some method of narcotisation, such as the gradual addition of dilute solutions of chloral hydrate, potassium sulphate-nicotine, etc., which may render the organism less sensitive to the action of the fixing agent. In some cases this has been found to work most admirably, while in others it has just as signally failed.

The organisms finally fixed, they are next to be washed in water or dilute alcohol, as may be found best, then stained by some one of the usual methods, dehydrated in the usual way, cleared by the use of some one of the oils—cedar, clove, etc., and finally mounted in balsam, or, if preferred, in glycerine or glycerine jelly, in which cases attention must be directed to the different preliminary operations necessary.

At each stage of preparation similar precautions must be observed as to transfer from one medium to another, as have been suggested in the original transfer from the water to the killing medium. With reasonable care this will not be found a difficult matter, though of course some loss must be expected. With material thus prepared, the work of permanently mounting is comparatively simple, and the results worth all the pains which may have been involved in the preparation. I have permanent preparations of such organisms as *Amœbam*, *Paramœcium*, *Volvox*, etc., in beautifully expanded conditions, and exhibiting almost every phase of life-history peculiar to the several forms.

CHARLES W. HARGITT.

Syracuse University, May 20, 1899.

## A Method for Extracting Air and Other Gases from Objects.

One often finds, after killing and hardening any small animal, that some part of it, commonly the intestine, contains air-bubbles. Also, in decalcifying small calcareous animals with acids, especially when one hurries the process, bubbles of CO<sub>2</sub> are not infrequently formed. At all events, whatever the origin of the bubbles, their presence is adverse to obtaining perfect sections and series.

Such bubbles of gas can be most readily removed by the following process. It is well known that water contains a considerable quantity of air and some other gases in solution, and that these can be removed by boiling the water. Boil, therefore, for a short time, several hundred cc. of water, and while this is still quite hot pour it into a bottle with a tightly-fitting stopper, leaving little or no air between the water and the stopper. When the water has become cooled to a lukewarm temperature, place the objects in question into the water and cork the bottle tightly as before. After several hours (two or three to twenty-four hours) the water will have absorbed all the gas and the object will have settled to the bottom. Should the object still contain gas, repeat the operation. Should one fear that the object suffer injury, normal salt solution, or alcohol, might be suggested. Unfortunately, however, alcohol contains but little gas in solution.

I have often used the above method with good success, as have others also, at my suggestion.

E. W. BERGER.

Johns Hopkins University.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

## IV.

## STAINING.

Staining is one of the most important and most complicated processes of microtechnique. The formulæ are largely empirical and there is still abundant room for experimentation in the use of mordants and in the effect of the same stain or combination after various fixing agents.

Stains may be classified in various ways; e. g., there are three great groups of stains—the Carmines, the Hæmatoxylin, and the Anilins. Stains may be classified as basic and acid, or they may be regarded as general and specific. A general stain affects all the elements, while a specific stain affects only certain elements or stains some elements more deeply than others. Stains which show a vigorous affinity for the nucleus have been called nuclear stains, and those which affect the cytoplasm more than the nucleus have been termed plasma stains. Of course, such stains are specific.

We shall consider some of the more important hæmatoxylin, carmines, and anilins, reserving general directions and theoretical questions for a subsequent paper. Many of the formulæ are taken from "The Microtome's Vade-mecum" (Lee), which is easily the most complete compendium of stains and other reagents concerned in microtechnique. It is to be regretted that botanists have no book of this character, but it must be confessed that we have not the material for such an extensive work. Other formulæ are from "Botanical Microtechnique" (Zimmermann), and from "Stirling's Histology." The directions for using a stain apply to stains made up according to the formulæ which are given here and may need modification if other formulæ are employed. It is hoped, however, that the directions will give the student sufficient insight into the *rationale* of staining to enable him to make any necessary modifications.

## THE HÆMATOXYLINS.

The most important hæmatoxylin is Delafield's hæmatoxylin, Kleinenberg's hæmatoxylin, Erlich's hæmatoxylin, Boehmer's hæmatoxylin, Mayer's hæm-alum, and Haidenhain's iron-alum-hæmatoxylin.

All the hæmatoxylin mentioned above contain alum, and according to Mayer, who has written the most important work on hæmatoxylin stains (Ueber das Färben mit Hæmatoxylin. Mitth. a. d. Zoöl. Station zu Neapel. 10: 170-186, 1891), "The active agent in them is a compound of hæmatin with alumina. This salt is precipitated in the tissues, chiefly in the nuclei, by organic and inorganic salts there present (e. g., by the phosphates) and perhaps also by other organic bodies belonging to the tissues." These salts are fixed in the tissues by the killing and fixing agent, and when the stain is applied a chemical combination results. Hæmatoxylin stain well after any of the fixing agents described in the preceding paper, but they are most effective when used after the chromic acid series of fixing agents.

*Delafield's Hæmatoxylin.*—"To 100 cc. of a saturated solution of ammonia alum add, drop by drop, a solution of 1 g. of hæmatoxylin dissolved in 6 cc. of absolute alcohol. Expose to air and light for one week. Filter. Add 25 cc. of glycerine and 25 cc. of methyl alcohol. Allow to stand until the color is sufficiently dark. Filter, and keep in a tightly stoppered bottle" (*Stirling and Lee*).

The solution should stand for at least two months before it is ready for using. This "ripening" is brought about by the oxidation of hæmatoxylin into hæmatin, a reaction which may be secured in a few minutes by a judicious application of peroxide of hydrogen.

Transfer to the stain from 35 per cent. alcohol or from water. The length of time required is exceedingly variable. Sometimes sections will stain deeply in three minutes, but it is often necessary to stain for thirty minutes. The length of time required will be fairly uniform for all material taken from the same bottle. This fact indicates that the washing process, which follows killing and fixing, is an important factor; if the washing has been thorough, the material will stain readily, but if the washing has been insufficient, the material may stain slowly or not at all. The washing is particularly important when the fixing agent contains an acid. Transfer from the stain to water. Distilled water is neither necessary nor desirable. Precipitates are often formed when slides are transferred directly to alcohol from this stain; otherwise, it would be better to transfer from the stain to 35 per cent. alcohol. Pass through the alcohols to 70 per cent. alcohol and then give the slide a few dips (two seconds is often sufficient) in acid alcohol. (1 cc. HCl. to 100 cc. of 70 per cent. alcohol.) This extracts the stain more rapidly from other parts than from the nuclei and hence gives a good nuclear stain. Some prefer to stain for a very short time and use no acid alcohol, but, as a rule, it seems best to overstain and then differentiate in this way. Transfer from acid alcohol to 70 per cent. alcohol and leave here until a rich purple color replaces the red due to the acid. Since small quantities of the acid alcohol are carried over into the 70 per cent. alcohol, it is well to add a *drop* of ammonia now and then to neutralize the effect of the acid. Too much ammonia is to be avoided, for it gives a disagreeable bluish color with poor differentiation, probably on account of the precipitation of alumina. The slide may now be passed through the alcohols, cleared in xylol, and mounted in balsam; or, if a double stain be preferred, treat for thirty seconds to one minute with eosin, erythrosin, or some other stain affording a good contrast; rinse in 70 per cent. alcohol and proceed as usual.

Delafield's hæmatoxylin is the most generally useful stain in the hæmatoxylin group. It brings out cellulose walls very sharply, and consequently is a good stain for embryos and the fundamental tissue system in general. With safranin it forms a good combination for the vascular system, the safranin giving the lignified elements a bright red color, while the hæmatoxylin stains the cellulose a rich purple. It is a good stain for chromatin and the achromatic structures show up fairly well, but can be brought out much better by special methods. Arche-spore cells and sporogenous tissue are very well defined if proper care be taken. Whenever you are in doubt as to the selection of a stain for general purposes, we should advise the use of Delafield's hæmatoxylin.

*Kleinenberg's Hæmatoxylin.*—This stain has had a wide use, but is now largely replaced by better formulæ. It is mentioned here merely because it is on the shelves of so many laboratories. It is doubtful whether it is equal to Delafield's in any kind of botanical work. A good description is given in the Quarterly Journal of the Microscopical Society, **74**: 208 —, 1897.

*Erllich's Hæmatoxylin.*—

Distilled water, 50 cc.  
Absolute alcohol, 50 cc.  
Glycerine, 50 cc.  
Glacial acetic acid, 5 cc.  
Hæmatoxylin, 1 gr.  
Alum in excess.

Keep it in a dark place until the color becomes a deep red. If well stoppered, it will keep indefinitely. Transfer to the stain from 50 per cent. or 35 per cent. alcohol. Stain five to thirty minutes. Since there is no danger from precipitates and the solution does not overstain, it is not necessary to treat with water or with acid alcohol, but the slide may be transferred from the stain to 70 per cent. alcohol. Eosin, erythrosin, or orange G are good contrast stains.

*Boehmer's Hæmatoxylin.*—

A { Hæmatoxylin, 1 gr.  
Absolute alcohol, 12 cc.  
B { Alum, 1 gr.  
Distilled water, 240 cc.

The solution A must ripen for two months. When wanted for use, add about 10 drops of A to 10 cc. of B. Stain ten to twenty minutes. Wash in water and proceed as usual.

*Mayer's Hæm-alum.*—Hæmatoxylin, 1 gr., dissolved with heat in 50 cc. of 95 per cent. alcohol and added to a solution of 50 gr. of alum in a liter of distilled water. Allow the mixture to cool and settle; filter; add a crystal of thymol to preserve from mould (*Lee*).

It is ready for use as soon as made up. Unless attacked by mould it keeps indefinitely. Transfer to the stain from water. It is seldom necessary to stain for more than ten minutes, and four or five minutes is generally long enough. As a rule, better results are secured by diluting the stain (about 1 cc. to 10 cc. of distilled water) and allowing it to act for ten hours or over night. This is a good stain for the nuclei of filamentous algæ and fungi, since it has little or no effect upon cell walls or plastids. Wash thoroughly in water and transfer to 10 per cent. glycerine. (See Vol. 1, No. 9 of this JOURNAL.) Specimens may be mounted in balsam if they can be gotten through without shrinking.

*Haidenhain's Iron-Alum-Hæmatoxylin.*—This stain was introduced by Haidenhain in 1892 and has gained a well deserved popularity with those engaged in cytological work. Two solutions are used and they are never mixed.

A.  $1\frac{1}{2}$  to 4 per cent. aqueous solution of ammonia sulphate of iron. (At present we use a 3 per cent. solution.)

B. One-half per cent. aqueous solution of hæmatoxylin.



The first solution acts as a mordant, i. e., it does not stain, but prepares the tissue for the action of the second solution.

Transfer to the iron-alum from water; allow this solution to act for two hours; wash in water five minutes and then stain in the one-half per cent. hæmatoxylin ten hours or over night. Rinse in water five minutes and treat for a second time with the iron-alum, which now rapidly extracts the stain. The action of the iron-alum should be watched under a microscope, and when the chromosomes of karyokinetic figures appear sharply defined, the slide should at once be placed in water and washed for at least an hour, since a very little of the iron-alum, if left in the tissue, will cause the preparation to fade. If staining for details other than nuclei, the slide must be transferred to water as soon as the desired effect is produced. After the washing in water, the slide is passed through the alcohols, cleared in xylol, and mounted in balsam. This stain is excellent for the filamentous algæ and fungi and it keeps well in glycerin. For preparations to be mounted in balsam, erythrosin, fuchsin, or orange G make good contrast stains. Apply the second stain after the last washing in water. The second stain should always be very light.

Chromosomes and centrosomes ("of those plants which have centrosomes") take a brilliant black and other details, though not brightly colored, often show excellent definition.

The times given above must not be accepted as final. Many prefer to wash in water for several hours after the first immersion in iron-alum. A plan which has proved convenient and very successful is to put the slide into the iron-alum in the morning, let it wash in water during the afternoon, leave it in the one-half per cent. of hæmatoxylin over night, and finish the preparation the next morning. It is a long process, requiring care, patience, and judgment, but it is worth the effort.

#### THE CARMINES.

This group of stains, immensely popular a few years ago, has rapidly lost favor among botanists as newer stains and combinations have appeared. When it is desirable to stain in bulk, nothing has been found which will serve better than the carmines. Only three of these stains will be considered.

##### *Greenacher's Borax Carmine.*—

Carmine, 3 gr.

Borax, 4 gr.

Distilled water, 100 cc.

Dissolve the borax in water and add the carmine, which is quickly dissolved with the aid of gentle heat. Add 100 cc. of 70 per cent. alcohol and filter (*Stirling*).

Stain from 50 per cent. alcohol, twelve to twenty-four hours; treat with acid alcohol five to ten minutes. After the paraffin has been dissolved out with xylol, the preparation may at once be mounted in balsam, or it may be counter-stained with any stain which forms a good contrast.

*Alum Carmine.*—A 4 per cent. aqueous solution of ammonia alum is boiled twenty minutes with 1 per cent. of powdered carmine. Filter after it cools (*Lec*).

Stain from water twelve to twenty-four hours and wash in water. No acid alcohol is needed since the solution does not overstain.

*Alum Cochineal.*—

Powdered cochineal, 50 gr.

Alum, 5 gr.

Distilled water, 500 cc.

Dissolve the alum in water, add the cochineal, and boil; evaporate down to two-thirds of the original volume, and filter. Add a few drops of carbolic acid to prevent mould (*Stirling*).

Stain as with alum carmine. A few years ago it was a very common practice to stain in bulk in alum cochineal and counterstain on the slide with Bismark brown.

#### THE ANILINS.

Many of the most brilliant and beautiful stains yet discovered belong to this group. These stains are so numerous that we shall not attempt to mention even their names, but shall consider only those which are in most common use by botanists. The following formula has proved to be fairly satisfactory for all the anilins mentioned in this account, but other formulæ will be given for most of the stains:

Make a 3 per cent solution of anilin oil in distilled water; shake well and frequently for a day; add enough alcohol to make the whole mixture about 20 per cent. alcohol; add 1 gr. of cyanin, erythrosin, safranin, gentian violet, etc., to each 100 cc. of this solution.

The anilins keep well in balsam, but not in glycerine. Xylol is a good clearing agent for all of them, but clove oil very much better in case of gentian violet. Unfortunately, some of them do not give permanent stains. Some are acid, some basic, and some neutral.

The rapidity with which sections must be transferred from one fluid to another makes them more difficult to manage than the hæmatoxylin or the carmines, but the stains are so valuable that even the beginner should spend most of his time with the anilins.

Many anilins stain quite deeply in three to twenty minutes, but if the stain washes out during the dehydrating process, stain longer, even ten to thirty hours if necessary. If the stains are made up according to the formula mentioned above, transfer to the stain from 35 per cent. alcohol and from the stain to 35 per cent. alcohol if the stain does not wash out too rapidly; if the stain washes out, try 50 per cent., 70 per cent., 85 per cent., 95 per cent., or even directly to absolute alcohol. It will often be found impracticable to transfer from the stain to alcohols weaker than the 85 per cent.

Since the anilins are seldom used as single stains, but almost invariably in combination with other stains, the logical order will be disregarded and the stains will be treated, as they are used, in their most usual combinations.

*Cyanin and Erythrosin.*—Stain in cyanin ten to thirty minutes or longer; rinse quickly in alcohol and then stain thirty seconds to one minute in erythrosin. If the cyanin washes out, stain for an hour, and if it still washes out, omit the rinsing in alcohol and transfer directly from the cyanin to the erythrosin.

This is a good combination for general work, and if properly used is excellent for mitotic phenomena and the most delicate cytological work. Delafield's hæmatoxylin may be used with erythrosin. Stain first with the hæmatoxylin, and after the purple color has replaced the red due to the acid, stain lightly with erythrosin. Eosin may be used instead of erythrosin, but is less transparent. Eosin will be mentioned later in connection with special methods for algæ and fungi.

*Flemming's Safranin-Gentian Violet-Orange.*—Safranin has long been a famous stain for karyokinesis. This triple combination was published in 1891, but its value in plant cytology was not thoroughly appreciated until five or six years later, when its application was developed to a high degree of perfection by various investigators of the Bonn (Germany) school.

According to Flemming, stain two to three days in safranin (dissolve 0.5 gr. safranin in 50 cc. absolute alcohol, and after four days add 100 cc. distilled water); rinse quickly in water; stain one to three hours in a 2 per cent. aqueous solution of gentian violet; wash quickly in water, and then stain one to three minutes in a 1 per cent aqueous solution of orange G. Transfer from the stain to absolute alcohol, clear in clove oil, and mount in balsam.

The following method seems to be better for mitotic phenomena in plants: Transfer to safranin from 35 per cent. alcohol and stain sixteen to twenty-four hours. If the stain acts for only a few hours, it washes out too rapidly to be controlled with any precision. (The safranin may be made up according to the formula given in the preceding paragraph or according to the general formula.) Rinse in water for a minute and then in 50 per cent. alcohol until only nucleoli and the chromosomes of dividing nuclei retain the red color. If the alcohol does not wash out the stain sufficiently, add a few drops of hydrochloric acid (not more than 0.1 cc. HCl. to 100 cc. alcohol.) If acid has been used, wash for a moment in pure 50 per cent. alcohol and then stain in gentian violet (aqueous solution, or made up according to the general formula). Rinse for a few seconds in water and then stain about thirty seconds in a 1 per cent. aqueous solution of orange G. Transfer from the stain directly to absolute alcohol and hasten the dehydrating by gently rinsing the slide in the fluid. As a rule, not more than ten seconds can be allowed for dehydrating because the gentian violet washes out so rapidly. Treat with clove oil for five to ten seconds. The clove oil not only clears, but it rapidly extracts the gentian violet, producing an elegant differentiation. Replace the clove oil by cedar oil. Cedar oil does not extract the stain. The preparation should now be examined under a microscope, and if the stain is still too deep the clove oil may be applied a second time. Mount in balsam. If mounted directly from clove oil, the gentian violet is almost sure to fade. Chromosomes should take a clear red and the spindle fibers a bright violet. This combination works best after Flemming's solution, but does fairly well after other members of the chromic acid series. Achromatic structures do not seem to stain well after corrosive sublimate or picric acid.

*Fuchsin.*—Use a 1 or 2 per cent. solution in water or in 70 per cent. alcohol. Transfer to the alcoholic solution from 70 per cent. alcohol; stain one to two hours; differentiate the stain in 1 per cent. solution of picric acid in 70 per

cent. alcohol—this may require thirty seconds or several minutes; <sup>5</sup>/<sub>10</sub> rinse in 70 per cent. alcohol until a bright red replaces the yellowish color due to the acid, and then proceed as usual.

*Iodine Green.*—A 1 per cent solution in 70 per cent alcohol is good for the vascular system of plants. This stain resists the washing-out process better than methyl green. Stain in iodine green at least an hour, and it is not a bad plan to stain over night; rinse in 70 per cent. alcohol; stain 15 seconds to one minute in erythrosin, and proceed as usual. Methyl green may be made and used in the same way.

*Fuchsin and Iodine Green Mixtures.*—Two solutions are kept separate, since they do not retain their efficiency long after they are mixed.

- |   |   |                            |
|---|---|----------------------------|
| A | { | 0.1 gr. fuchsin (acid).    |
|   | { | 50 cc. distilled water.    |
| B | { | 0.1 gr. iodine green.      |
|   | { | 50 cc. distilled water.    |
| C | { | 100 cc. absolute alcohol.  |
|   | { | 1 cc. glacial acetic acid. |
|   | { | 0.1 gr. iodine.            |

Mix equal parts of A and B. Transfer to the stain from water. The proper time must be determined by experiment. Transfer from the stain directly to solution C and from C to xylol.

Another formula :

- A. 0.5 gr. acid fuchsin.  
B. 0.5 gr. iodine green.

Mix a pipette full of A with a pipette full of B; stain two to eight minutes; transfer to 85 per cent. or 95 per cent. alcohol, dehydrate rapidly, clear in xylol, and mount in balsam. Both these formulæ are good for karyokinesis.

*Bismark Brown.*—Use a 2 per cent. solution in 70 per cent. alcohol. If material has been stained in bulk in one of the carmines, a few minutes staining on the slide with Bismark brown gives a good contrast. It is particularly good for cell walls.

*Nigrosin.*—Use a 1 or 2 per cent. solution in water. A few drops of this solution to a watch glass full of water stains filamentous algæ or fungi in one to three hours. The stain keeps well in glycerine or balsam, but it is hard to get these forms into balsam without more or less shrinking.

(To be Continued.)

If it is inconvenient to use a pipette for drawing off the water from a watch-glass containing small organisms, a couple of threads dipping over the side will serve the purpose.

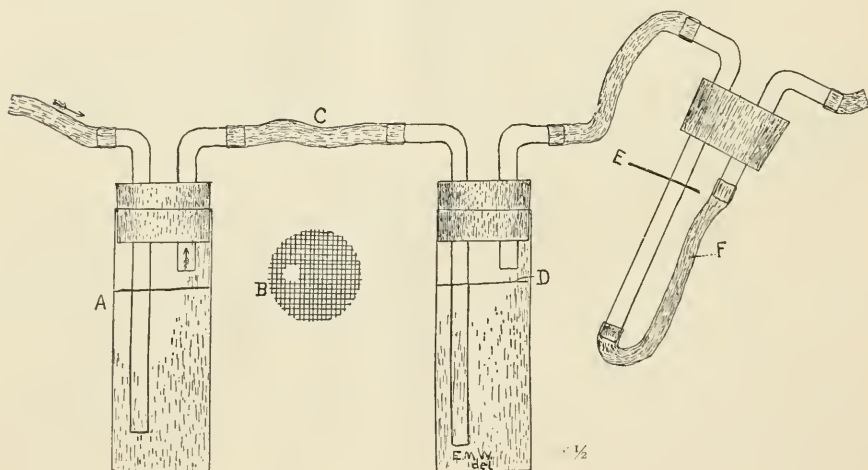
Hydra, worms, and other animal forms that it is desired to kill fully distended may be stupefied by gradually adding small amounts of rochelle salts to the water containing them. The time required varies; it sometimes takes two or three days. The animals become relaxed and do not contract when treated with the fixing agent.



## A Convenient Washing Apparatus.

The apparatus here described and figured is especially adapted to the use of several students in one laboratory. By its use a large amount of material may be freed of chromic acid, or other acid mixture, in a very short time.

Above the sink are placed several shelves, with holes in each to hold a series of six or more vials. To each student is assigned a given number of these vials. Each vial is provided with a rubber stopper holding an inlet and outlet tube. The former tube reaches to within a centimeter of the bottom, while the latter reaches but the same distance below the stopper. A piece of copper gauze, cut to fit the vial, is inserted on the long tube (B and D) about a centimeter below the outlet tube. In place of the gauze one can use a similar piece of perforated sheet copper. The material is placed below this gauze and is thus prevented from entering the mouth of the outlet tube.



When the material is thoroughly washed the vial may be removed and the tubes connected by rubber tubing (F). In this way any vial may be removed without interfering with the use of the remainder of the vials in the series. It is advisable to connect a small pipe to the main water pipe back of the spigot, so that the general use of the water is not prevented. In this smaller vertical pipe should be placed small spigots on the level of the several shelves. The vials on each shelf may then be used independently of the others. A common pipe carries the waste water to the sink.

On one shelf can be arranged a series of Coplin staining jars, connected in the same manner as the vials. The two tubes in this case scarcely project from the bottom of the stopper, while just below them a circular piece of copper gauze is placed on the four projecting edges of the jar. Sections stained on the slide can be quickly washed in such an apparatus.

E. MEAD WILCOX.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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LOCAL microscopical societies are productive of a great deal of good, both to their members and to science at large, and there ought to be one in every community large enough to support a half dozen physicians and a high school. The expense of such an organization need not be greater than is desired by the members, as a fixed meeting place is not necessary, though desirable. The good which comes of meeting together, and each receiving the benefit of the others' experience, can only be told by those who have tried it.

\* \* \*

A pedagogical museum is to be established at the University of Texas for the purpose of furnishing instruction in the university regular courses, and in the courses of the university summer school, to those who are fitting themselves to be teachers. The collections will be classified as follows:

- a.* Plans of school buildings, of heating and ventilating apparatus, school furniture, and decorations for school rooms.
- b.* Maps, charts, globes, models, and all scientific apparatus; supplies required for demonstration and laboratory work in mathematics, physics, chemistry, botany, zoölogy, physiology, etc., in grammar and high-school courses.
- c.* Kindergarten material and primary aids.
- d.* Manual training tools and benches.
- e.* Sets of text-books.
- f.* Pedagogical works.

The primary object of this museum is to bring together these various objects and the apparatus required in the equipment of a school, and to introduce them to the notice of teachers in such a manner as to give them an idea not only of what may be had for the work which they will be called upon to do, but the proper methods for the use and care of it. This is a long step in advance and one which ought to bear abundant fruit in Texas. The excellent example of the University of Texas ought to be followed in every institution which sends out men and women to teach the sciences. Familiarity with apparatus and skill in its manipulation are fully as important factors in the successful teacher's equipment as knowledge of the principles and minutiae of biology, for without them effective demonstration is difficult, if not impossible. It is a sorry painter who can not mix his own colors, and a teacher who is not the master of those means of demonstration required in the laboratory, and who does not enter into every detail of the construction, equipment, and business conduct of the laboratory, or that part of it over which he presides, can scarcely be expected to do justice to himself or his pupils.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Durand, E. J.** A Washing Apparatus. Bot. Gaz. 27: 394-395, 1899. This seems to be the best of the many devices for washing material which has been fixed in such solutions as chromic acid or Flemming's chromo-aceto-osmic mixture. It consists of a tin box supported on legs, and provided with a cork bottom (insect cork at least 1 cm. thick) through holes in which are passed glass tubes, drawn out to a point at the lower end. The size described is 6.25 cm. deep, 8.75 cm. wide, and 29.5 cm. long. The sides and ends are turned in 5 mm., to support the cork bottom, which is further supported by four narrow metal crosspieces. The tubes are 6.75 cm. long, with an inside diameter of 3 mm. A box of this size is large enough for eighteen tubes. Stretch a fine meshed cloth over the mouth of the bottle, and keep it in place by a rubber band. Force the pointed end of the tube through the cloth, and turn on water just sufficient to cause a gentle circulation. C. J. C.

**Lind, K.** Ueber das Eindringen von Pilzen in Kalkgesteine und Knochen. Jahrb. f. wiss. Bot. 32: 603-604, 1898. It has long been known that many algæ, fungi, lichens, and bacteria are able to penetrate into calcareous rocks and bone. Herr Lind, a dentist, has made a series of experiments, from which he concludes that the penetration is due in great part to chemical irritation. He used thin plates of marble, only one-fifth of a millimeter in thickness. When the plate is moistened on one side with a nutrient solution, and spores are sown on the other side, the mycelium penetrates the plate, but if both sides are moistened or both sides kept dry, no penetration occurs. Plates of chalk and bone were used with similar results. The corroding power, in many cases, is due to carbonic and oxalic acid secreted by the plant. Decay of teeth is due to bacteria. Particles of food clinging to the teeth give rise to acids which corrode the enamel, and thus allow the bacteria an entrance, after which they further the decalcifying, and finally, by their peptonizing power, destroy even the organic substance of the teeth. As soon as the dentine is reached, the bacteria complete very rapidly the work of destruction.

The article contains a résumé of previous literature, and is illustrated by three wood cuts. The work was done under Pfeffer, at Leipzig. C. J. C.

**Nemec, B.** Ueber die karyokinetische Kernteilung in der Wurzelspitze von *Allium cepa*. Jahrb. f. wiss. Bot. 33: 313-336, pl. 3, 1899. In this account of the division of the nucleus of *Allium Cepa*, Nemec says that the achromatic spindle is not formed primarily as a multipolar affair and later becoming bipolar, as described for other plants by Osterhout, Mottier, and others, but that it begins in the bipolar form. Threads which appear just outside the nuclear membrane are soon seen to be

drawn out into poles at opposite sides of the nucleus. The spindle fibers of either pole may be of unequal length, but all converge toward a common point. Some of the figures suggest that other interpreters might claim for them the condition of multipolarity. The nuclear membrane does not often disappear until the achromatic spindle is almost formed, and there is no evidence justifying the statement that it contributes to the formation of the spindle. The form of the spindle is determined by the dimensions of the dividing cell. The development of the spindle in cells of the perleome shows characteristic differences from the same process in cells of the perileme.

At about the time the nuclear membrane disappears the chromatin thread breaks up into a few divisions, and these break again, forming the chromosomes. If the chromosomes are short they are placed parallel to the spindle threads, while if they are long there is no regularity in their position. Some spindle fibers pass directly from pole to pole, while others are attached to the chromosomes. The chromosomes split longitudinally, beginning at the looped end. After the pairs of chromosomes have divided somewhat, fibers are seen connecting them, and Nemec states that these fibers are entirely different in structure from those which first formed the spindle. They are thicker, more granular, and stain more like chromatin than the earlier formed fibers. As to their function, he says any statement would at present be a mere guess, but suggests that they may, by exerting a push, assist the fibers which are pulling the chromosomes.

Structures which have been described by some other writers as centrosomes are represented in the drawings, but the author thinks they are nucleoli, since they react toward stains as nucleoli, and are finally enclosed within the nuclear membrane.

The statement is made that the chief morphological difference between the division of cells of vegetative tissue, and those of sporogenous tissue, consists in the fact that the spindle in divisions of the former is always bipolar, while that of the latter may be multipolar at the beginning, and may or may not become bipolar.

Throughout the paper few references are made to work done by others along these same lines; and while the work is very similar, and in some cases a repetition of that done by Schaffner on *Allium Cepa*, and published by him in the latter part of last year, no reference is made to this work.

OTIS W. CALDWELL.

Chicago.

**Goldflus, Mlle. Mathilde.** Sur la structure et les fonctions de l'assise épithéliale et des antipodes chez les Composées. Journ. de Botanique, 12: 374-384, 1898: 13: 9-17, 49-59, 87-96. 6 pls., 1899.

The author has made an extensive study of the embryo-sac and surrounding tissues in composite ovules, from a point of view which, while not new, is unusual.

It is well known to all students of composite ovules that the embryo-sac, before maturity, ruptures the nucellus, and, continuing its growth, pushes the heavy integument away from the funiculus, forming for itself a spindle-shaped cavity. This cavity is, of course, lined by the epidermis, and the cells bordering upon the sac, having a perfectly columnar form, were called *endodermis* by Hegelmaier, and *endothelium* by Schwere. Mlle. Goldflus has suggested the term



*epithelium* as being more appropriate. She sees in this layer a digestive layer, as did Guignard, rather than a layer for the protection of the sac, as Hegelmaier suggested.

The antipodal cells are found to vary in number, form, and the number of nuclei which they contain. They are believed to be partly digestive and partly conductive in function. Their densely staining protoplasm, and their tendency in some species to enlarge and burrow back into the chalazal region (we see no reason for adopting the term pseudo-chalaza) are cited in support of this view.

It will not be calling in question the soundness of the author's conclusions if it be suggested that the view of many botanists, following Strasburger, that the antipodals are, morphologically, a prothallial region, need not be set aside upon the discovery that they have acquired a special physiological function. Certainly, no one would claim that the so-called epithelial layer ceases to be a part of the epidermis because it has, in many cases, become glandular.

A strong point in the author's favor is found in the sharp differentiation of the cells of practically all the ovules studied, into an outer region of flattened cells, presumably to protect the growing embryo, and an inner, fan-shaped region, whose cells, elongated radially, all converge toward the sac. That this region serves to conduct nutritive materials to the sac, is hardly to be doubted. As the embryo grows, the "digestion" of the cells of the ovule begins in this region, along the sides of the sac, while a strand of elongated cells is left behind the sac, forming almost a direct path from the end of the vascular bundle to the antipodal cells. This last fact, by the way, while it argues in favor of the conductive function of the antipodals, argues just as strongly against any digestive activity on their part.

It is unfortunate that the figures which accompany this important paper are unsatisfactory. The text figures are too small to show cell details, and the plates represent the sacs and epithelial cells somewhat diagrammatically, and show but a few of the surrounding cells. A few ovules, carefully drawn with their embryo-sacs, would have made a valuable addition to the paper.

W. D. MERRELL.

Chicago.

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## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
Agnes M. Claypole, Sage College,  
Ithaca, N. Y.

## CURRENT LITERATURE.

**Czermak, N.** Ueber die Desintegration und die Reintegration des Kernkoerperchens bei der Karyokinese. *Anat. Anz.* **15**: No. 22, pp. 414-430, 1899.

The author presents in this paper some observations on the nucleolus with reference to the changes it undergoes during the various phases of nuclear

activity. The work was undertaken to test an earlier statement, made by the author in 1895, "that all elements pass through an integrating process resulting in an arrangement in groups and masses, to be later scattered and more or less mixed with each other." This has been demonstrated for the chromosomes during karyokinesis, and these studies were planned to determine the action of other cell elements. As material, the salamander testis and segmenting salmon egg were used, and the results of the work, though not quite complete, are presented in this paper.

In summarizing the present status of the question, it is found that according to Rhode on nerve cells the nucleoli are clearly a thickening of the nuclear network. Small parts separate off and wander, with a part of the nuclear substance, into the cell body, become surrounded by a nuclear membrane, and eventually form a new nucleus and cell. This nucleolus is here the prime mover in cell division. Hæcker and Reichert, in fertilization among copepoda, maintain that the nucleolus lies in the nuclear sap in stages when the chromosomes are fully formed, and later vanishes, from which it can be concluded that it plays no important part in the karyokinesis. Metzner asserts that in the spermatogonia of the salamander the nucleolus divides into many smaller ones, and these scatter through the cell and apparently reassemble in the midst of the daughter skein and grow to their former size. Korschelt says that in new blastomeres of *Ophryotrocha* the nucleolus appears as a spherical mass of chromatin. When the nucleus prepares for a new division, the nucleolus enlarges and acquires a clearly alveolar structure, staining darkly. The chromatin threads and nucleolus both reach their greatest development at the same time. The nucleolus now begins to degenerate; it becomes paler and thinner, and separates into granules on the periphery, and eventually appears as a sphere filled with pale granules, the result of the destruction of its network. After some of the achromatin part of the division spindle appears, the nucleolus is still visible in the nuclear cap.

Omeltchenko, investigating the pancreas, liver, testis and kidney of the rabbit and guinea pig, found a rod-like structure which stained in Biondi with two blue spherical ends and an intermediate rose-colored ellipse. Longitudinal splitting is followed by loss of the outer membrane and fibrous structure of the masses, which become thicker and now stain uniformly blue, and form a

chromatic thread. Later this undergoes all the changes seen in the karyokinesis, and in the stages of the daughter skein the central chromosomes become the nucleolus and the peripheral the nuclear membrane.

Lukjanow describes the follicle cells of the testis of the intestinal parasite of the dog, and says: The nucleolus consists typically of three parts, a middle part which, on double staining with hæmatoxylin and safranin, takes the red stain and appears ellipsoidal. Two markedly larger end pieces stain a dark or violet red. J. Wagner, in studying the spermatogenesis of the spider, describes two kinds of nucleoli, one large and genuine, and two smaller and false. The first divides independently of the chromosomes, and often not at the same time; the second take part in the formation of chromosomes.

Strasburger, in botany, studying the centrosome and spindle formation, distinguishes in protoplasm two kinds of substance, thread-like "kinoplasm" and alveolar "trophoplasm." In recent publications he considers the nucleolus as a reserve material for the formation of the spindle and the nuclear membrane.

Mitzkewitsch, studying karyokinesis in spirogyra, says that the whole mass of chromatin is in the form of a spheroidal nucleolus. At the beginning of karyokinesis uneven staining power is shown, chromosome granules appear, and a surrounding pale substance, containing fibers that pass out into the colorless threads of the protoplasm. The darkly staining part forms the chromosomes, and the fibrous part the spindle of the karyokinetic figure. On a return of the resting state, the nucleolus reappears from the chromosomes and spindle threads.

Czermak himself found earlier that the resting nucleus in the segmenting egg of the salmon contained a very large spherical nucleus. Fixation was principally in a mixture of one-fourth per cent. chromic acid, one-half per cent. acetic acid, and one per cent. platinum chloride, with many others also. Staining was according to Fleming, or with Haidenhein's iron hæmatoxylin. After Fleming's orange stain and overstaining with gentian, the nucleoli were almost black, the chromosomes violet, the yolk granules red, and the protoplasm golden yellow. Shortly summarizing his results, the author finds that the nucleoli in the material studied disintegrate into (chromatin granules) an oxychromatic network and a dark substance (in the salmon), and also take part in the formation of chromosomes, and probably the spindle fibers. The disintegration appears to go on so that at first an oxychromatic substance rises, probably, from the fibers of the spindle in which the chromatic substance and probably the dark material afterwards is absorbed.

A. M. C.

**Hoffmann, Dr. R. W.** Zur Orientirung kleinster mikroskopischer Objecte. Zeits. f. wiss. Mikr. 15: 312-316, 1899.

A mixture is used consisting of equal parts of clove oil and collodion, which is left in a wide-mouthed bottle in an exposed place for twenty-four hours. Afterward the mass is placed in xylol, where it becomes transparent. Objects in this mass are placed on glass slips about two, or two and one-half cm. long, by one-half, or three-quarters cm. wide, and oriented by means of currents produced by drawing a needle through the mass. The glass slip gives a perfectly level surface, and the stained specimen may be seen as clearly in this medium as a *toto* mount in balsam. Orientation

lines may be scratched on the glass, if desired. The same mixture serves as an imbedding medium. The collodion plate containing the oriented specimen is carried into xylol for a short time, then left in paraffin for about five minutes, until the xylol adhering to the glass is absorbed.

For specially difficult orientation the specimen may be placed in clove oil and collodion under a cover-glass, with two thin tubes for rollers, and rolled into position. A drop of ether will thin the medium, which may then be left to reach such a consistency that the object cannot be turned, before placing in paraffin. Sections may be cut four or five  $\mu$  thick.

The method is valuable for small objects and for objects that are not easily penetrated by paraffin, or that would be injured by heat, or where there is much yolk or albumen and the specimen is liable to crumble when sectioned.

E. M. BRACE.

**Stokes, Dr. Alfred C.** Fresh-Water Infusoria. The life cycle of the infusoria is described, and there are descriptions and illustrations of several forms, with directions for collecting them. In examining them, the writer uses a simple life-slide, which consists of a shallow cell made of shellac, with two opposite portions scraped away to admit the air, and covered with a square cover-glass (Fig. 1). A modification of Logan's life-box (Fig. 2)

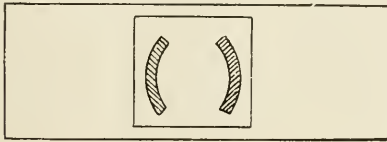


Fig. 1—Life-cell.

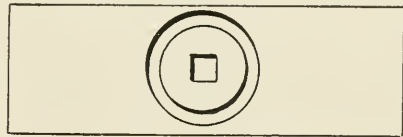


Fig. 2—Life-box.

is made by cementing a small cube of glass to a slide with canada balsam. This cube is surrounded with a thick glass or zinc ring having a layer of wax on it to which a cover-glass is cemented by running a hot needle around the edge. The life-box should be air-tight to prevent the evaporation of the drop of water containing infusoria, which is placed on the central cube.

E. M. BRACE.

**Lécaillon, A.** Recherches sur l'oeuf et sur le développement embryonnaire de quelques Chrysomélides. Thèses présentées à la Faculté des Sciences de Paris. Sér A. No. 299, 1-219, 4 pls., 2 figs., 1898.

The author has studied six of the chrysomelidæ, *Cytra læviuscula*, *Gastrophysa raphani*, *Chrysomela mentastri*, *Lina populi*, *L. tremulæ*, and *Agelastica alni*. It seems there is no chitin present in the envelopes of these eggs, and segmentation is of the typical, superficial form usual to insect eggs. The author objects, however, and very wisely, to the term "superficial," and suggests "intravitelline" as more descriptive of the process, since the egg nucleus segments in the middle of the egg and the nuclei move outward through the yolk mass afterwards. Those reaching the surface form ectoderm, while those remaining behind are to produce entoderm. Mesoderm rises from the ectoderm, and is very variable in its manner of appearance. Sex cells arise very early and are ectodermic, while the whole alimentary tract is formed from the proctodæum



and stomatodæum; hence there is no entodermic portion. In these points the author corroborates the work of Heymons.

A. M. C.

**Tichomirow, A.** Zur Anatomie des Insectenhoden. Zool. Anz. 21: 623-630, 5 figs., 1898.

The testis of the silk moth consists of connective tissue stroma and germ cells.

The stroma is arranged to form a sheath with a septa, and between there is a delicate network of connective tissue strands subdividing the cavities formed by the septa. "Verson's" cell appears above each of the four chambers as a localized spot in the connective tissue bridges, where the tissue is richly supplied with plasma. On later maturation of the spermatocytes a connective tissue cell becomes transformed into a Sertoli cell, giving clear evidence on the different tissue origin of this much discussed structure from that of the spermatozoa.

A. M. C.

**Faussek, V.** Ueber die Ablagerung des Pigmentes bei Mytelus. Zeitschr. f. wiss. Zool. 65: pp. 112-142, 3 figs., 1898.

The Lamellibranch *Mytilus* was principally used in this investigation, and the most important conclusion is that pigment formation is entirely independent of light.

By removing one of the shells it was found that light caused no access of pigmentation to the exposed surface, but the controlling factor seems to be the amount of oxygen present in the water flowing over the surface. By changing the direction of the water flow so that it washed the anterior part of the body before it passed over the posterior, pigment was formed in the anterior, normally colorless region. The author concludes that pigmentation is due to the oxidation of a chromogen present in the blood, and therefore the part of the body having the largest amount of oxygen supplied to it is most deeply pigmented.

A. M. C.

**Meek, A.** Further note on the Post-Embryonal History of Striped Muscles in Mammals. Anat. Anz. 15: pp. 474-466, 1899.

This is a reply or criticism of the work done by Dr. B. Morpurgo on the muscular tissue in white rats. (Reviewed

in Journal of Applied Microscopy, February, 1899.) The present author published a preliminary report on some studies containing diametrically opposite results in one respect. (Anat. Anz. 14: p. 619, 1898.) In the present reply he adds several further observations. The point in question is the post-embryonic multiplication of muscle fibers. By reference to Morpurgo's paper it will be seen that in white rats there is, after birth, mitotic division of the muscle elements, and hence an actual increase in the number of fibers; two succeeding stages follow, amitotic increase in the number of nuclei, and increase in the size of the fibers. Meek had before demonstrated a reduction in the number of fibers after birth. His observations have been carried on in the cat, sheep, and field vole (*Arvicola agrestis*). His results in each case showed a reduction in numbers of fibers, and hypertrophy of those remaining. Several different muscles from different parts of these animals were examined, also some from the white rat, and the results were uniform. The author admits that considering the very immature condition of the latter at birth, there may be a continuance of certain foetal processes after birth in some muscles. The reduction he finds occurs more markedly in females than males, and while in young individuals the two

sides of the body are practically alike, differences appear later. The author considers his method of determining numbers more reliable than Morpurgo's. The latter made drawings, while Meek, beginning with this method, abandoned it and actually counted the fibers in the squares of an eyepiece micrometer, and added the totals; in both cases sections of tissue were used. The inequality of size of fibers is considered to be due to degenerative changes, caused by the intra muscular "struggle for existence and survival of the fit." Exercise will hasten the results of the struggle, while rest and feeding will delay it. The author will soon publish a complete discussion of his results. A. M. C.

**Vernon, H. M.** The Relations Between Marine Animal and Vegetable Life. Mitth. Zool. Stat. zu Neapel. 13: pp. 341-426, 1898.

This investigation was carried on especially to determine the nature of the cycle of changes through which nitrogenous material excreted into the water may undergo, and also as to the effects of aeration. Chemical, physiological, and bacteriological questions were introduced, and the study carried on along these lines. Larvæ of the sea urchin *Strongylocentrotus lividus*, readily obtainable from artificial fertilizations, were grown in water of various kinds, and after eight days growth were killed, preserved, and microscopically examined with reference to size, in sets of fifty; the mean size of the sets from different tanks was then taken as a basis of comparison. The chemical side consisted in making determinations of the free and organic or albuminoid ammonia present in the various specimens of water. The method used was that of Wanklin and Chapman, and consisted in distilling half a liter of water in question and collecting it in volumes of 50 or 100 cc.; 4 per cent. of Nestler's reagent is added, and the brown color resulting is determined by a standard. For the amount of ammonium chloride, after distillation of 200 cc. of the 500, all free ammonia is removed, and treating with potassium permanganate and distilling off 150 cc. more, the organic ammonia is separated. Bacteriological tests were made on gelatin plates, careful count being kept of the colonies found. It was determined that such weeds as *Ulva* rapidly remove free ammonia, but add organic ammonia to tank water; larvæ living under these conditions usually increase in size. Red weeds, as *Gelidium*, usually cause an increase in both forms of ammonia, and as a rule are unfavorable to larval growth. Filtration of the water through sand removes almost all free and two-thirds of the organic ammonia and favors larval development. Keeping water in the dark for three or more weeks results in the removal by bacterial action of nearly all the free, and a third or more of the organic ammonia; larvæ grown under these conditions are larger than usual. Ammonium chloride acts injuriously to larval life, but potassium nitrite and nitrate have no effects. Aeration has only a very slightly favorable effect on larval growth. A. M. C.

**Von Linden, M.** Untersuchungen ueber die Entwicklung der Zeichnung des Schmetterlingsfluegels in der Puppe. Zeit. f. wiss. Zool. 65: pp. 1-50, pls. 1-3, 1898.

The writer has carried on the work with special reference to three points: first, as to whether the marking of the imago appear suddenly in the pupa, or gradually; second, as to whether there is evidence in the ontogenetic development of these markings, of the phylogenetic history of the form studied;

thirdly, as to whether the ontogenetic development of these wing characters shows a definite series of developmental processes, both as to markings and to the series of colors. Investigations show in reference to the first question that butterfly markings are in general put together from a series of elements that appear successively during the development of the pupa, and are only combined a short time before the butterfly matures. This gradual development of markings shows most clearly in forms of lower phylogenetic standing; higher developed forms show temporarily the more primitive kinds of marking. The first markings to appear are of generic value. The results of this investigation confirm most thoroughly Eimer's generalizations on the relationship of the Papilionidæ. It was found that there is a marked distinction to be made between the ground color and the markings. The longitudinal markings represent the first steps in ontogeny as in phylogeny. First, small longitudinal veins appear, which later fuse laterally, and finally are united by transverse markings. Among the Van-essas, *Vanessa levana* shows the most primitive features; here longitudinal markings are most strongly expressed. The coloring of the wings and the fusion of the veins are first completed on the back and inner parts, and development spreads from these regions forward and outward; edges and ribs last receive their definite coloring in all cases. In many instances the hind wing developed more rapidly than the fore, and the upper side than the under. The position and course of the veins are dependent on the shape of the wing, hence the imaginal markings of the two wings vary widely from originally similar beginnings. In single cases the position of the veins is dependent on the course of trachea, and it is probable that the origin of the primitive, longitudinal venation of the butterfly could be traced back to a neuropterous arrangement. It was found that colors appear in the following order: first, light yellow, then orange, carmine, zinnober, and lastly, black. Blue appears after black.

A. M. C.

**Van Beneden, Edward.** Sur la présence, chez l'homme, d'un canal archentérique. *Anat. Anz.* 15: pp. 349-356, 9 fig., 1899.

Recent work by Eternod (*Anat. Anz.* 15: 11-13, 1898) on a young human embryo suggested to Van Beneden a

point of interest observed earlier by himself. It concerns the development of an archenteric canal in man. Its existence has not been observed, but its presence can be safely inferred from the facts presented. Eternod speaks of a solid rod of cells stretching along the whole length of the embryo, excepting in the region of the blastopore, where it is changed into a deep furrow, which the observer suggests may be the notocordal or archenteric canal in its early stages. Van Beneden points out that this embryo, and also another famous one of the Comte Spee, are both more advanced than would be expected for the beginning of this canal, and therefore states that this furrow is the last trace, and not first evidence of this structure. Figures are given of some sections of these young embryos, and some most interesting points brought out. Several new names are given to the different cell masses. The hypoblast is called the "lecithophore," the beginning of the neureuteric canal is named "enteric plate." Originally distinct in origin, these two eventually fuse to a single mass and form an indivisible plate known as the "lecitho-enteric plate." Close study of the sections gives

evidence to the fact that the archenteric canal for the greater part of its length opens into the blastodermic cavity, and hence is confounded with it, owing to the absorption of the "lecitho-enteric" plate, which is eventually split longitudinally. The author summarizes his results by stating that the archenteric canal appears as an early evagination from the archenteron, and, agreeing with other mammals, the notocordal plate is formed from certain cylindrical cells of the archenteric epithelium, not, as stated by Lieberskuehn and Koelliker, from the whole median expansion of the archenteron. Figure A, a cross section, shows on the median line immediately below the ectoderm a median plate of larger entodermal cells continuous at each end with the general entodermic layer, that is the notocordal plate. Below this is a small mass of cells which forms the floor of the archenteric canal called the "enteric plate." This takes no part in the formation of the notocord, but finally fuses with the entoderm or "lecithophore" below to form the alimentary entoderm or "lecitho-enterique" plate.

A. M. C.

**Guenther, A.** Untersuchungen ueber die im Magen unserer Hauswiederkæuer vorkommenden Wimperinfusorien. *Zeit. wiss. Zool.* **65**: pp. 529-573, 2 pls., 1899.

**Smith, G. Elliott.** Further Observations on the Anatomy of the Brain in the Monotremata. *Jour. of Anat. and Phys.* **5**: 33, 309-342, 2 pls.

**Smith, G. Elliott.** The Brain in the Edentata. *Transact. of the Linn. Soc. of Lond.* **7**: 277-394, 36 figs.

**Hesse, R.** Untersuchungen ueber die organe der Lichtempfindung bei niederen Thieren. 5, Die Augen der Polychæten Anneliden. *Zeit. wiss. Zool.* **65**: 446-516, 5 pls., 1899.

**Bonnet, R.** Gründriss der Entwicklungsgeschichte der Haussæugethiere, Russische uebersetz. v. G. J. Smetlow u. P. Schmidt, St. Petersburg, 278 pp., 1898.

**Brachet, A.** Die Entwicklung der grossen Kærperhæhlen und ihre Trennung von einander. (Perikardial,—Pleural und Peritonealhæhle). Die Entwicklung der Pleuro-Perikardialmembran und des Zwerchfells. *Ergebn. d. Anat. u. Entwicklungsgesch.* **7**: pp. 886-936, 11 figs., 1897 (1898).

**Stieda, S.** Bericht ueber die Anatomische, Histologische und Embryologische Literature Russlands (1896-1897). *Ergbn. v. Anat. u. Entwicklungsgesch.* **7**: 530-693, 1897.

**Dastre, A., et Floresco, N.** Contribution à l'étude des chlorophylles animales. Chlorophylle dufoie des Invertebrates. *Compt. Rend. Acad. d. Sc., Paris.* **128**: 398-400.

**Flemmling, W.** Morphologie der Zelle. *Ergebn. v. Anat. u. Entwicklungsgesch.* **7**: 403-585, 1897 (1898).

## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review to H. H. Waite, 710 East Catherine street, Ann Arbor, Michigan.

**Ravenel, M. P.** Anthrax—The Influence of Tanneries in Spreading the Disease

From reports which the author was not able to entirely verify, it is probable

that twelve men and sixty head of cattle died of anthrax in Pennsylvania during the year 1897. The men were, without exception, operatives from the tanneries, and the cattle were from pastures watered by the streams carrying off the refuse of these tanneries.

In one of the cases, in a man who subsequently recovered, virulent cultures of the anthrax bacillus were obtained from the blood and eschar which marked the initial lesion of the man then sick. Virulent anthrax bacilli were also



isolated from the liver and spleen of a cow that had died at one of the tanneries. In a bacteriological examination of one of twelve cows that had died some ten miles below one of the tanneries, the anthrax bacillus was not obtained, but the author believes that it was the cause of the disease from the symptoms described.

In tracing the history of these cases, it was ascertained that a part of the hides were shipped from China, and though there is a law in the United States prohibiting the importation of hides from districts where anthrax is known to exist, it is nevertheless difficult to obtain accurate data regarding the health of cattle from such remote districts. Cases are sometimes reported in workers in leather, and one case has been reported in Philadelphia, in a brush maker, who used hog's bristles and horse-tail hair in the manufacture of brushes. The hair used was imported from Siberia and the southern part of Russia, where anthrax occurs quite frequently.

Since all the cases, from which a positive history could be obtained, had occurred in those who had handled the hides before subjection to the process of tanning, experiments were made with anthrax bacilli. Silk threads were impregnated with a spore-culture of the germ and dried. The cultures used for this purpose were obtained from the organs of a cow that had died of anthrax. Since hides contain a large amount of albuminous material which combines with tannin, bacteria are in this way covered and protected, and hence are not as easily killed as they would be on silk threads.

After subjecting these threads to the tanning solution, cultures were grown from time to time from the threads, and inoculations were made in guinea pigs. Death occurred in from 24 to 36 hours. Two hundred and forty-four days after placing the silk threads in the tanning solution, or a period of time more than twice as long as is required in tanning, cultures were made from the sediment of the tanning solution. These cultures, obtained from spores which did not even have the protection of silk thread, killed guinea pigs weighing 470 and 500 grams, in 28 and 24 hours respectively after inoculation.

These experiments show that some method should be devised to disinfect the hides, both before and during the process of tanning. The author sums up his article as follows: For the protection of our cattle industry and of the operatives in tanneries, the points of importance are: "First—The discovery of an economic and simple method of thoroughly disinfecting hides without injury. Second—A more reliable method of obtaining reports from countries which export hides. Third—A process of tanning which will in itself destroy the spores of the anthrax bacillus."

H. H. W.

Smith, Dr. Erwin F. Gelatin Culture Media.  
Am. Nat. 33: 214-215, 1899.

Different individuals working with gelatin culture media may obtain varying results with the same organism. There may be a number of disturbing influences, among the most confusing of which are: (1) the fact that gelatin may be acid to phenolphthalein, while it is neutral or moderately alkaline to litmus, and often exerts a restraining influence on bacteria, especially certain parasites; and (2) the fact that cane sugar, or grape sugar in gelatin, while stimulating growth,

often prevents liquefaction so that one may be dealing with a liquefying organism without knowing it.

Gelatin media should be rendered neutral to phenolphthalein, and the per cent. of gelatin used, and the melting point of the prepared media, stated.

E. M. BRACE.

**Dorset, Dr. M.** A New Stain for Bacillus Tuberculosis. N. Y. Med. Jour., **69**: 148-149, 1899.

Sudan III may be used as a selective stain for tubercle bacilli. The preparations are treated with an 80 per cent.

alcoholic solution of the stain for five minutes or more, and the excess of stain washed out with 70 per cent. alcohol. If desired the preparations may be contrast-stained with methylen-blue.

E. M. BRACE.

**Money, C.** Methode zur Färbung der Bakterien in den Geweben. Centrblt. f. Bakt. **25**: 424, 1899.

The sections should first be stained in picro-carmin, borax-carmin, or alum-carmin, preferably in picro-carmin,

then in gentian-violet, or methylen-blue. Two or three drops of formalin should be added to each watch glass of the staining solution. The preparations should then be heated until vapor begins to be given off. The excess of stain in the sections is then washed off with water, and the sections decolorized in 90 per cent. alcohol. It is best not to leave the sections too long in the formalin gentian-violet solution, since they will decolorize slowly and with difficulty. With accurate technique, the author has obtained very good results by this method.

H. H. W.

**Symmers, Wm. St. C.** Report on Preparation of Plague Serum. Centrblt. f. Bakt. **25**: 460-464, 1899.

The author prepared his sera by inoculating horses with cultures of the bacillus pestis bubonicæ grown in

bouillon, and with bouillon suspensions of the bacilli grown on agar. In none of the horses was there a marked reaction with the exception of one, which died, and he attributes the death of this animal, not to the toxic effects of the material inoculated, but to penetration of a blood vessel and subsequent occlusion of an important vessel in the brain.

The serum was obtained from the horse in the usual manner by bleeding the animal from the jugular vein. Serum from one of the horses in quantities of one-quarter of a cubic centimeter was insufficient to save rats infected with the minimal fatal dose. Another horse produced a serum which, in quantities of one-quarter of a cubic centimeter, protected white rats from the minimal fatal dose of the bacillus, but a smaller amount of the serum was incapable of protecting the rats.

From these investigations the author draws the following conclusions:

1. The strength of the above sera is not sufficiently great to warrant any hope of their being of therapeutic use in an actual epidemic of plague.
2. The antitoxic power of the serum (obtained at Abbassieh) is equal to that prepared by Yersin, so far as can be gathered from his published writings.
3. Sera of about the same value were prepared by him at the Serum Institute of the British Institute of Preventive Medicine in the case of cholera and

typhoid fever. Hence it would appear that the method adopted in these three cases, cholera, enteric fever, and plague, does not afford sera sufficiently strong to be of any use in the treatment of these diseases.

4. Probably more severe inoculations, and the use of more virulent bacilli for a longer time, might afford a more powerful serum.

H. H. W.

**Strong, L. W.** A Study of the Encapsulated Bacilli. Jour. Boston Society Med. Sciences, Feb., 1899.

In his study of the capsule forming bacilli the author divides them into two classes, the Friedländer group and the ærogenes group. Friedländer's group comprises *Bacillus pneumoniae*, Friedländer, *Bacillus ozænæ*, Fasching, *Bacillus capsulatus mucosus* or *Bacillus sputigenus crassus*, bacillus Wright and Mallory, and possibly bacillus rhinoscleroma. From the results obtained the author is inclined to believe that all the above group might be assumed to be variations of a primary distinct organism, the *Bacillus pneumoniae*. The ærogenes group includes *Bacillus capsulatus*, Pfeiffer, *Bacillus kruse*, and *Bacillus ærogenes*, though there are probably other members of the group which were not studied. This group the author also believes should be regarded as originally having arisen from a primary distinct organism. The chief characteristics of the Friedländer group are: "bacilli forming primary colorless colonies, becoming whitish when old; easily stained capsules which occur only in tissues and exudates; pseudo-capsules, occasionally in artificial media; gas production, most abundant on saccharose, slightly less on glucose, and little or none on lactose; no, or slight, acid formation on lactose; and no coagulation of milk." The characteristics of the bacilli studied under the ærogenes group are: "colonies primarily whitish; capsules difficult to stain, and inconstant in occurrence; no pseudo-capsules in artificial media; more abundant and constant gas formation on all three media; rapid coagulation of milk, and equal amounts of acid formation on all three sugars." Gas production and acid formation furnish valuable information for their study and identification.

H. H. W.

**Jaos, A.** Untersuchungen über Diphtherie-diagnose. Centrblt. f. Bakt. 25: 296-304 and 351-357, 1899.

This work covers a series of investigations with the various media employed in the diagnosis of diphtheria. Of all the methods formerly employed, he finds Löffler's blood serum the most satisfactory, though this at times he finds has serious objections, since colonies of other bacteria, notably streptococci and staphylococci, develop, which may render the recognition of the diphtheria bacillus difficult, and at times these colonies may be so numerous as to cover up entirely the diphtheria colonies, and thus lead to error in diagnosis. With these objections in view, the author has prepared media of different composition, and has finally adopted one on which the diphtheria bacilli readily develop, and the streptococci and staphylococci not at all or very poorly. The composition and preparation of the medium with which he obtained the best results is as follows: 300 cc. of ordinary blood serum is mixed with 50 cc. of a normal sodium-hydrate solution and 150 cc. of distilled water or bouillon. This mixture is placed in a flask with a flat bottom and heated over a water-bath for two or three hours at a temperature of from 60 de-

tions with the various media employed in the diagnosis of diphtheria. Of all

grees to 70 degrees C. The temperature is then raised to 100 degrees C. or, what is still better, the flask is placed in a steam sterilizer for from one-half to three-quarters of an hour. To this mixture is added an equal volume (500 cc.) of peptonized bouillon and 20 g. of agar, which is dissolved as quickly as possible. As soon as the solution is complete it is filtered while hot and sterilized for one-fourth of an hour at 100 to 110 degrees in an autoclave and then poured into sterile Petri dishes.

The preparation of this medium is quick, easy, and demands no especial care. Precaution should be taken, however, not to bring the mixture too quickly to a high temperature, since the albumin of the serum would become coagulated. Time must be allowed, with gentle heat, to combine the alkali with the albumin. This union of albumin and alkali can also be brought about by placing the mixture (blood serum, sodium-hydrate, and water or bouillon) in the incubator for several days. Frequently there is formed in this mixture a flocculent sediment which is not dissolved by heat. This sediment has no significance, and consists principally of insoluble salts formed by the action of the sodium-hydrate on the bouillon. It is not necessary that the serum used for this nutrient medium should be sterile, since it is sterilized before use at a temperature of 110 degrees C. The advantages which this medium offers and which are not combined in any other medium are:

1. The preparation of the serum-agar is simple, easy, and quick.
2. This serum-agar is a very definite, accurately known compound which can be kept constantly on hand with the greatest ease. The results obtained with it in the various laboratories can, therefore, be accurately compared with one another.
3. The cultures can be made in Petri dishes. The substances used in inoculation can be so distributed over the surface of the medium as to obtain widely separated colonies. Since the medium is quite transparent and contains little color, the cultures can be examined under a microscope with a magnification of 60 or 70 diameters.
4. Diphtheria bacilli always develop on serum-agar. Several hundred parallel diagnoses have been made with cultures on Löffler's blood serum and on serum-agar, and the latter has never failed. The method used by the author, therefore, establishes an absolutely sure diagnosis.
5. The diagnosis can frequently be made in five to six hours, always after keeping the cultures in the incubator twelve to fifteen hours.
6. The examination of the cultures is less troublesome than by any other method, since the confusion due to the growth of various other colonies is done away with. The diphtheria colonies, even when they are very small, can be very easily recognized, since their appearance is always typical. The streptococci do not develop on this medium, and the growth of the staphylococci is materially inhibited.

H. H. W.



## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Pathological Laboratory, Boston City Hospital, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

### CURRENT LITERATURE.

**Jenner, Dr. L.** New Preparation for Rapidly Fixing and Staining Blood. *Lancet*, **1**: 370-371, 1899.

Equal parts of a 1.2 per cent. solution of Grüber's water-soluble eosin, yellow shade, and a 1 per cent. solution of

Grüber's medicinal methylen-blue, are thoroughly mixed by stirring with a glass rod, and filtered after twenty-four hours. The residue is dried at a temperature not exceeding 55 degrees C., powdered, washed on a filter with distilled water, dried, and again powdered.

The fixative stain is made by dissolving 0.5 g. of this preparation in 100 cc. methyl alcohol. Cover-glass preparations are made by allowing a few drops of the solution to act on the dry film for from one to three minutes. Then pour off the stain, rinse in distilled water from five to ten seconds, or until the films become pink, and dry the cover-glass, preferably in air. E. M. BRACE.

**Clark, J. G., M. D.** The Origin, Growth, and Fate of the Corpus Luteum, as observed in the Ovary of the Pig and Man. *John Hopkins Hospital Reports*, **7**: 4, 1898. Also *Archiv. für Anat. und Physiol. Anat. Abtheilung*, 1898.

There are two theories in regard to the development of the corpus luteum. According to v. Baer's theory, the one more commonly accepted, this body is derived from the cells of the theca

interna. According to Bischoff's theory, the lutein cells are modified cells of the membrana granulosa.

From the ovary of the pig, Clark first obtained a complete set of specimens representing all changes, from the maturity of the Graafian follicles to complete organization and disappearance of the corpus. With these he compared the changes in the human ovary.

In order to study the relations of the connective tissue he used a digestion (trypsin) method. The principal stain used was a modification of Van Gieson's picro-fuchsin stain.

His results agree with those of v. Baer. The lutein cells are specialized connective tissue cells which appear in the follicle wall at the time of its differentiation into the theca interna and externa. The corpus luteum is therefore a connective tissue, and not an epithelial, structure. The lutein cells are formed at the expense of the ordinary connective tissue which forms a fine reticular tissue between the cells and also produces the membrana propria. Upon the rupture of the follicle the membrana propria is broken through, in places, by the advancing lutein cells and blood vessels, but quickly reforms a connective tissue line in front of the lutein cells. This is pushed by them to the center of the cavity, where it forms a dense core of interlacing fibers.

After rupture of the follicle the lutein cells, by increase in size and number, fill the cavity. The degeneration which follows is probably induced by the

increasing density of the surrounding connective tissue. This degeneration consists in a fatty degeneration of the lutein cells, followed by a shrinking of the connective tissue network into a compact body, the corpus fibrosum. This body finally undergoes hyaline changes, leaving a fine scar tissue, which may be entirely lost in the ovarian stroma. The blood vessels are the last structures to give way.

The function of the corpus luteum is to preserve the ovarian circulation. This it does by preventing not only the persistence of the dilated vessels surrounding the unruptured follicle, but also by preventing the too rapid formation of scar tissue. In the young woman this function is well performed; but in older women, with increasing density of the stroma, the circulation is gradually impaired.

Cessation of ovulation is induced, not through the disappearance of the follicles, but through the increased density of the stroma, which impairs the circulation and prevents the development of the follicles.

The article is illustrated by reproductions of stained and digestion preparations.

R. M. P.

**Mallory, F. B.** A Histological Study of Typhoid Fever. *Jour. Exper. Med.* 3: 6, 1898.

Mallory has made a very complete histological study of nineteen cases of typhoid fever with the object of throw-

ing some light on the primary essential lesions of typhoid fever, and also on certain secondary lesions which result therefrom. The essential lesions he believes to be a proliferation of the endothelial cells. This proliferation is due to the action of the toxin of the typhoid bacillus, the toxin being produced not only in the intestinal tract, but also in the blood and organs of the body. The cells, which are markedly phagocytic in character, are produced most abundantly along the line of absorption from the intestinal tract in both the lymphatics and the blood vessels. They are also produced in the vessels of the general circulation as the result of the general distribution of the toxin, and finally in the lymphatic spaces of the body generally as the result of the absorption of the toxin eliminated from the blood vessels.

The swelling of the intestinal lymphoid tissue, of the mesenteric lymph nodes, and of the spleen is due almost entirely to proliferation of the endothelial cells. The phagocytic power of these cells is very marked. The cells most frequently devoured are lymphoid cells, occasionally red blood corpuscles, and less often plasma cells or polynuclear leucocytes. In some cells ten to twenty lymphoid cells could be counted. A diffuse increase of these cells is also found in the mucous membrane of the intestine generally. There is also an increase of plasma and lymphoid cells.

The necrosis in the intestinal lymphoid tissue is due to the occlusion of the veins and capillaries by fibrin thrombi, which owe their origin to the degeneration of phagocytic cells beneath the lining endothelium of the vessels.

The necroses in the liver are due to obstruction of the liver capillaries by phagocytic cells derived chiefly by embolism through the portal circulation. These cells originate from the endothelium of the blood vessels of the intestine and

spleen. The liver cells lying between the occluded capillaries undergo necrosis and disappear. The phagocytic cells themselves become necrotic, and fibrin forms between them. Focal lesions due to phagocytic cells may occur also in the kidney, heart, and testicle. The origin of the lesions in these three organs is not yet definitely determined.

The various processes are illustrated by eight colored plates, showing thirty-four figures.

R. M. P.

**Robertson, W. Ford.** On a New Method of Obtaining a Black Reaction in Certain Tissue Elements in the Central Nervous System (Platinum Method). *Scottish Med. and Surg. Jour.* 4: 1, 1899.

The tissue is placed in twenty times its volume of a 5 per cent. formalin solution with 1 per cent. platinum chloride. The bottle is loosely corked

and the tissue is allowed to remain in the fluid for several weeks after the tissue begins to blacken, which it generally does in one to three months. If the platinum chloride becomes exhausted before blackening is marked, more can be added. Sections are cut with the freezing microtome and mounted as in other impregnation methods.

The platinum chloride picks out and blackens certain elements, among which are long branching and anastomosing fibers in the walls of the blood vessels (arteries, capillaries, arterioles, and venules) of the cord and brain. Robertson believes these to be the connective tissue fibers of the adventitia.

When the reduction process is more marked the primitive fibrils of the nerve cells are darkened. The Nissl bodies cannot be seen. Around the nucleus of a nerve cell the primitive fibrils can be seen forming a network, and it is from this network of fibrils that the axis cylinder is formed. In the nucleus of the large nerve cells are seen six to twelve large blackened particles, evidently the acidophilic particles.

In the brain of the dog were found, in both cortex and medullary substance, numerous small branching cells with a round nucleus and a small amount of granular protoplasm. The nucleus is about the size of a red blood corpuscle; the processes are three to six in number. These do not appear to have any relation to other histological elements of the brain. Similar cells were seen in the one human brain examined by this method. Robertson thinks that these cells form a special tissue element of the nervous system not previously recognized as such.

R. M. P.

**Kronthal, P.** Eine neue Färbung für das Nervensystem. *Neurologisches Centralblatt.* March 1, 1899.

This stain for the ganglion cells and fibers of the central nervous system depends on the precipitation of lead

salts. Pieces of tissue are placed in equal parts of a 10 per cent. formalin solution and a saturated solution of lead formate for five days, and then in equal parts of 10 per cent. formalin and a saturated solution of hydrogen sulphite for five days, after which they are placed in alcohol until imbedded. All the cells and the fibers, to their finest ramifications, are sharply impregnated.

R. M. P.

**Harris, H. F., M. D.** Ripening Hæmatoxylin. Prof. Harris, acting upon a suggestion made by Paul Mayer, has found that mercuric oxide (red or yellow) readily oxidizes hæmatoxylein into hematein. He prepares the solution as follows:

1 g. hæmatoxylin xtals, dissolved in 10 g. absolute alcohol.  
20 g. ammonium or potassium alum, dissolved with heat in 200 cc. distilled water.

Mix the two solutions at once, or better, after twenty-four hours, and add one-half gram of mercuric oxide; heat to boiling, and cool quickly. The liquid may be used at once for staining. If a precipitate forms later, the solution may be filtered. The addition of chloral to some of the staining fluid gave satisfactory results.

E. M. BRACE.

## NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA MCL. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

**Viola, C.** Ueber Feldspathbestimmung. Zeit. f. Kryst. 30: 23-35, 1898.

The well-known method of Michel-Lévy\* in which microlitic feldspar crystals are determined by measuring the extinction angles in easily recognized zones, and ascertaining the maximum angle, is modified by Viola as follows:

It is assumed that cross sections of *all* orientations are equally likely to occur in a hap-hazard rock section, and do occur, with equal frequency.

But it is not true that all values of extinction angle between the limits of the species are equally liable to occur. For example, in anorthite, in the zone normal to 010, the extinction angles range from 0 to nearly 50 degrees, but an extinction of 0 to 5 degrees can occur through 2½ degrees, whereas an extinction of 40 to 45 degrees can occur through 19 degrees—that is, an extinction of 40 to 45 degrees is about eight times as likely to occur, in sections from the zone normal to (010), as an extinction of 0 to 5 degrees.

The probabilities are calculated in this way for each extinction angle in each zone, then the most probable and least probable angles are tabulated. A number of sections in the desired zone are measured, the extinctions arranged in order and compared, and the conclusion drawn.

For example, in the zone [100] the probabilities are:

		Most Probable Extinction.	Least Probable Extinction.
<i>Ab</i>	Albite,	4° to 6° and 18° to 20°	0° and 13° to 15°
<i>Ab<sub>9</sub></i>	<i>An<sub>2</sub></i> Oligoclase,	0° to 4°	
<i>Ab<sub>11</sub></i>	<i>An<sub>3</sub></i> Andesite,	4°	0° and 5°
<i>Ab<sub>7</sub></i>	<i>An<sub>3</sub></i> Labradorite,	0° and 18° to 19°	13° to 15°
<i>Ab<sub>2</sub></i>	<i>An<sub>3</sub></i> Labradorite,	0° and 31° to 32°	18° to 26°
<i>An</i>	Anorthite.	56°	28° to 48°

\*Étude sur la détermination des Feldspaths dans les plaques minces. Paris, 1894.



In a rock from Ticchiena, Rome, thirteen measurements in the zone [100] gave :  
0, 1, 1, 2, 2, 4, 13, 16, 16, 17, 17, 18, 19 degrees.

Five are near zero, six are near 18, one is at 13—that is, it conforms to Labradorite  $Ab_2 An_3$ .

**Viola, C.** Versuch einer elementaren Feldspathbestimmung in Dünnschliffe nach dem allgemeinen Principe der Wahrscheinlichkeit. Zeit. f. Kryst. 30: 36-54, 1898.

**Florence, W.** Darstellung mikroskopischer Krystalle in Lothrohrperlen. Neues Jahrb. f. Min. 1898, 2: 102-146. After describing a method for determining the *degree* of solubility (not the ease) by using a bead made on a loop

of 2.5 mm. diameter, with wire 0.3 mm. thick, and dissolving therein weighed or measured amounts of substance up to the saturation point, the author describes at length and illustrates with twenty-six photo-prints the method of obtaining crystals in blow-pipe beads.

The fluxes chosen are  $(KNa)_2 B_4O_7$ , the borate of potassium and sodium, and  $Na PO_3$  or salt of phosphorus. An almost spherical bead is made in a circular loop 3 mm. diameter, and *this is nearly saturated by lead oxide*,  $PbO$ , thus lowering the melting point and getting rid of disposable boracic or phosphoric acid. After this the substance is added in fine powder up to the point at which continued cloudiness indicates separation. The lead oxide assists by lessening the number of crystals formed, and also by lowering the fusion point so that the bead may be easily kept liquid and the incipient crystals given a chance to come together.

The hot bead is then pressed flat between two thick glasses and examined with the microscope.

The results are given for twenty-three oxides and twenty-four minerals; the resulting crystals and their optical behavior are described, as well as the bead colorations.

A. J. M.

**Daly, Reginald A.** A comparative study of Etch-Figures. The Amphiboles and Pyroxenes. Proc. Amer. Acad. Arts and Sci. 34: 373-430, March, 1899.

It is now well recognized that the little angular cavities or pits produced by the attack of a liquid or gas upon the face of a crystal, are of great assistance to the mineralogist in determining the symmetry of a crystal. The very elaborate study of the etch-figures of amphibole made by Mr. Daly was undertaken primarily, however, from a petrographical standpoint in order to find a means of orienting amphibole cleavages in crystalline rocks. It is of interest, also, as an attempt to devise a standard method from which all variables, except that of time, have been eliminated.

The method, in brief, consists in treating prominent faces, especially those parallel to cleavages, in concentrated commercial water solution of hydrofluoric acid, at the temperature of the water bath, until the figures developed almost overlap. A platinum crucible of about four cm. diameter at the bottom is filled to a depth of about one cm. with the hydrofluoric acid; the crystal is suspended in this in a platinum net, and the crucible placed in the water bath. The attack is stopped at any moment by plunging the net into cold water.

By maintaining these standard conditions it was believed that not only would

the etch-figures indicate the symmetry of the crystal, but that the *shape* of the etch-figures and their orientation with respect to cleavage lines would be essentially constant for the same crystal face in different crystals of the same compound.

The figures were examined by reflected, vertically incident light, with relatively low powers, and the general shape was determined by measuring the angles between straight sides, and their relation to some fixed base line as a crystal edge or a cleavage.

All the non-aluminous amphiboles gave essentially the same figure upon the prismatic face or cleavage, the differences being so minute as to defy measurement. The hornblende crystals yielded etchings upon the prism face, the main characters of which were constant, but in which four subtypes could be distinguished.

In etching the clinopinacoid of actinolite two quite different kinds of figures were developed, small, light, four-sided figures elongated parallel to the side most oblique to the cleavage, and larger, dark, four-sided figures elongated parallel to the side most nearly parallel to the cleavage. When the strength of the acid was varied the light pits were unchanged, but the dark pits were rotated about 30 degrees for a change from pure acid to 10 per cent. acid. All these characters showed similarity to Baumhauer's apatite.

In comparing amphibole and pyroxene, the author points out that actinolitic amphiboles give one class of etch-figures, aluminous amphiboles another; that there is a similar contrast between diopside and augite, and that the etching phenomena of diopside with hydrofluoric acid are hardly distinguishable from those of actinolite.

A. J. M.

**Sohncke, L.** Einfluss der Entwässerungstemperatur auf die Verwitterungsflecke des Gypses. Zeit. f. Kryst. 30: 1-8, 1898.

This paper well illustrates the careful attention that is, in these days, given to phenomena of apparently minor importance. Cleavage plates of gypsum, when heated, develop spots, some of which Pape described\* as of elliptical shape and composed wholly or in part of the dehydrated material. Blasius and Weiss were unable to obtain the elliptical spots, but Weiss described almost rectangular spots resembling an envelope, with four arms nearly diagonal. It is these envelope-like spots which Sohncke has examined.

The gypsum cleavages were heated in an air bath, either being suspended therein by a wire or laid upon asbestos paper. The spots were examined under the microscope, and were found to consist of four branching lines starting not exactly from a common center, nor quite at right angles. The ends of these determine a quadrilateral of which they are approximately the diagonals. When perfectly developed the quadrilateral is crossed by fine stripes parallel to two of the sides and nearly parallel to the conchoidal fracture, and in the triangles opposite the obtuse angles of the diagonals there appears another set of stripes parallel to the fibrous fracture.

Denoting the dimension of the figure parallel to the first set of stripes by  $l$ ,

\*Pogg. Ann. 125, 113, 1865; 133, 364, 1868.

and that at right angles thereto by  $q$ , it was found that the ratio  $\frac{q}{l}$  decreased steadily with an increasing temperature, that is, the value of  $l$  increased more rapidly than that of  $q$ , so that the spots, which after thirty-five minutes at 105 degrees were slightly longer in the direction  $q$ , ( $\frac{q}{l} = 1.180$ ), became after ten minutes at 112 to 118 degrees slightly longer in the direction  $l$ , ( $\frac{q}{l} = .882$ ), and after only one minute at 160 degrees were much longer in direction  $l$ , ( $\frac{q}{l} = 0.667$ ).

The stripes are about 2.7 to 3 degrees from parallelism to the conchoidal fracture, and make an angle of about 39 degrees with one diagonal, and 42 degrees with the other; the direction at 42 degrees to the stripes is approximately the direction of greatest thermal expansion in the cleavage plane. A. J. M.

## INDIVIDUAL SPECIES.

**Andalusit**, Ueber den, vom Montavon in Vorarlberg. H. Gemböck. Neues Jahrb. f. Min. 2: 89-98, 1898.

**Baddeckite**. A new variety of Muscovite. G. H<sub>4</sub> (Ca, Mg, K<sub>2</sub>, Na<sub>2</sub>) (Fe<sub>2</sub> Al<sub>2</sub>)<sub>3</sub> C. Hoffmann. Am. Jour. Sci., 4, 6: 274, Si<sub>8</sub> O<sub>28</sub>. A hydro-muscovite, with the usual proportion of alumina replaced

by ferric oxide. Occurs near Baddeck, Nova Scotia, in copper-red scales, which fuse to shiny black slag, and finally become magnetic. G.=3.252. Decomposed by strong hydrochloric acid. L. McI. L.

**Brookits**, Umwandlung und secundäre Zwillingbildung des, vom Rio Cipó, Brasilien. E. Hussack. Neues Jahrb. f. Min. 2: 99-101, 1898.

**Corundum**. On the origin of, associated with the Peridotites in North Carolina. J. H. Pratt. Am. Jour. Sci., 4, 6: 49, 1898. Author agrees with other investigators in regarding the peridotites as plutonic igneous rocks, and considers the corundum to have formed at the same time as the dunite, being held in solution by the molten mass of the dunite, and crystallizing out among the first minerals as the mass began to cool. The corundum is associated with clinoclhor. Many field evidences cited by author in drawing his conclusions, as well as results of experiments on solubility of alumina in molten basic glass, with separating out of corundum and spinel crystals on cooling. L. McI. L.

**Diaphorite** from Montana and Mexico. L. J. Spencer. Am. Jour. Sci., 4, 6: 316, 1898. Forms of crystals of this rare mineral recorded for these localities. Interesting morphotropic relation given between andorite, diaphorite, and freieslebenite, which suggests that the composition may also be proved to fall between that of andorite and freieslebenite. L. McI. L.

**Erionite**, a new Zeolite. A. S. Eakle. Am. Jour. Sci., 4, 6: 66, 1898. Found on milky opal as tufts of snow-white, woolly threads; or compactly matted in fissures. Lustre pearly. Fuses easily and quietly to clear, colorless glass. Yields much water. Soluble with great difficulty in hydrochloric acid. G.=1.997. Composition H<sub>2</sub> Si<sub>6</sub> Al<sub>2</sub> Ca K<sub>2</sub> Na<sub>2</sub> O<sub>17</sub> + 5 H<sub>2</sub> O, analogous to stilbite, with calcium largely replaced by alkalies. Complete optical determinations not made, but appears orthorhombic. Occurs in rhyolite-tuff from Durkee, Oregon. L. McI. L.

**Hardystonite**, a new Calcium-Zinc Silicate from Franklin Furnace, New Jersey. John E. Wolff. Proc. Am. Acad. Arts and Sci., 34: 479-481, 1899.

In a fine-grained mass of willemite, rhodonite, and franklinite at North Mine Hill, Franklin Furnace, were found grains of a white mineral for which the formula  $(\text{Zn Mn})\text{O}$ ,  $2(\text{Ca Mg})\text{O}$ ,  $2\text{SiO}_2$ , or  $\text{ZnCa}_2\text{Si}_2\text{O}_7$ , has been calculated. Cleavage and optical characters indicate the tetragonal system.  $G.=3.395-3.397$ ;  $H.=3$  to  $4$ ; lustre, glassy; color, white to translucent. Fuses with difficulty to a cloudy glass, gives an intense red flame. On coal glows and yields a heavy zinc coat. Optically negative; cleavage, evidently basal; shows the axial cross and two sets of rectangular cleavages evidently parallel to the first and second order prisms. Apparently an intermediate silicate near ganomalite.

A. J. M.

**Kalgoorlite**. E. F. Pittman. Records Geol. Survey, N. S. Wales, 5.

$\text{HgAu}_2\text{Ag}_6\text{Fe}_6$ . Massive, with sub-conchoidal fracture and iron-black color.  $G.=8.791$ . Occurs at the telluride deposits of Kalgoorlie, West Australia, associated with a yellow telluride of gold referred to calaverite.

L. McI. L.

**Krennerite** from Cripple Creek, Colorado. A. H. Chester. Am. Jour. Sci., 4, 5: 375, 1898.

Small, brilliant, pale yellowish-bronze crystals, occurring in quartzite gangue. Tin-white on common basal cleavage. Orthorhombic, with prismatic faces deeply striated, crystals resembling some forms of arsenopyrite. Crystallographic determination by Penfield;  $H.=2.5$ . Composition  $\text{AuTe}_2$  with 0.46 per cent. Ag. First occurrence noted outside of original locality. Author regards it as probable that calaverite will be found to be simply a silver free form of sylvanite.

L. McI. L.

**Native Lead**, with Roebbingite, Copper, etc., at Franklin Furnace, New Jersey. Warren M. Foote. Am. Jour. Sci., 4, 6: 187, 1898.

Found very sparingly in scales, nuggets, and films. No crystals. Analogy presented to occurrence at Langban, Sweden.

L. McI. L.

**Melanotekite** of Hillsboro, New Mexico; also note on composition of Kentrolite. C. H. Warren. Am. Jour. Sci., 4, 6: 116, 1898.

common type of basic silicate formula.

Author makes axial ratio,  $a:b:c=0.6338:1:0.9126$ , and composition  $(\text{Fe}_4\text{O}_3)\text{Pb}_3(\text{SiO}_4)_3$ , conforming to a Forms of crystals also recorded.

L. McI. L.

**Miersite**. Nature, April 14, 1898.

Name given by L. J. Spencer to a new form of silver iodide (isometric), found at Broken Hill, New South Wales. Occurs in cubes showing tetrahedral faces, and with dodecahedral cleavage. Pale or bright yellow color, with adamantine lustre.

L. McI. L.

**Rhodolite**, On the Associated Minerals of W. E. Hidden and J. H. Pratt. Am. Jour. Sci., 4, 6: 463, 1898.

Authors give detailed notes on quartz, quartz pseudomorphs, corundum, spinel group, bronzite, iolite, staurolite, monazite and zircon, cyrtolite, gold and sperryllite, found in the gravel beds of Mason Branch river, Macon county, N. C.

L. McI. L.



**Sperryllite**, Occurrence of, in North Carolina. W. E. Hidden. Am. Jour. Sci., 4, 6: 381, 1878.

Found in nugget-like masses and cubo-octahedral crystals, with rounded edges (not by abrasion), in old alluvial gravel

with gold, etc. Occurrence of this rare mineral widespread in vicinity of Caler Fork, Cowee Creek, Macon county. Not found *in situ* yet, but indications point towards its discovery along the pyritous belt of western North Carolina.

L. McI. L.

**Tantalite**, Crystallized, from Paris, Me. C. H. Warren. Am. Jour. Sci., 4, 6: 123, 1898.

Material not well adapted for crystallographic study, but forms of crystals recorded. Contains iron instead of the manganese found in previously described crystals.

L. McI. L.

**Tapiolite**, Crystallized from Topsham, Me. C. H. Warren. Am. Jour. Sci., 4, 6: 121, 1898.

Forms of crystals recorded. Essentially a tantalate, and contains very little niobium.

**Torbernite**, The Crystalline Symmetry of. T. L. Walker. Am. Jour. Sci., 4, 6: 41, 1898.

Cleavage plates, parallel to the perfect basal cleavage, show two other fairly good cleavages (about at 90°), which however are not equally perfect, as should be the case if the mineral were tetragonal. These basal cleavage plates show, in addition, mono-symmetric corrosion forms, which are symmetrical to the plane of second-best cleavage, and also biaxial optical character with very small axial angle.

Author concludes that torbernite, as well as antunite, is monoclinic (not tetragonal), and that the uranite minerals form an isomorphous group.

L. McI. L.

**Zircon**, Twinned Crystals, from North Carolina. W. E. Hidden and J. H. Pratt. Am. Jour. Sci., 4, 6: 323, 1898.

Small gray to reddish-brown crystals, well developed and doubly terminated, showing a series of twins with twinning planes parallel to pyramids of first order (new to zircon). Five new twinning planes identified. Found at Meredeth Freeman Zircon Mine, Henderson county.

L. McI. L.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace, Biological Laboratory, University of Rochester, Rochester, N. Y.

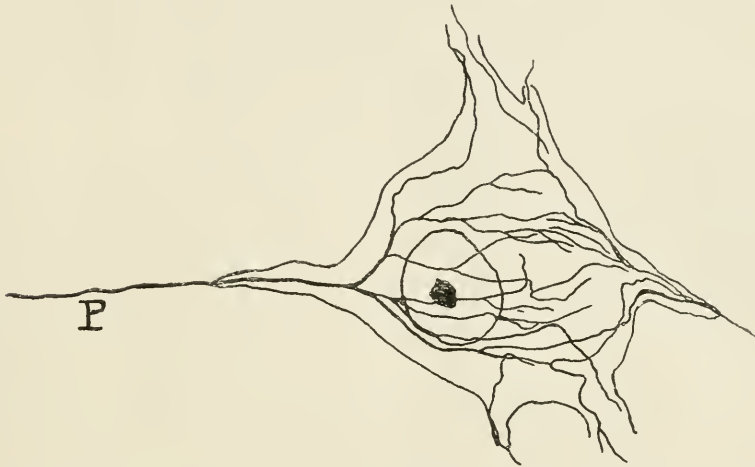
**Apáthy, S.** Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen. Mittheil. aus der Zoöl. Station zu Neapel. 12: 495-749, Pls. 23-32, 1897.

Investigations were made upon the nervous system of *Hirudo*, *Lumbricus*, *Helix*, and other invertebrates, and upon the vertebrates *Lophius*, *Rana*,

*Triton*, and *Lepus*, the chief stains used being gold chloride, methylen-blue, and hæmatein I A. He shows the fibrillar nature of the conducting substance, and the continuity of the conducting primitive fibrils which are traced through

ganglion, sense, and muscle cells. In this he contradicts the teachings of Bütschli, Leydig, and others, and asserts that "*nur Max Schultze und seine Anhänger haben Recht in Betreff der feineren histologischen Natur der Nervenfasere.*"

Each nerve cell produces a number of elementary fibrils, and these unite to form primitive fibrils, which divide themselves among the branches of the cell



Multipolar motor cell of *Lumbricus*. P. Primitive fibril formed by union of the neurofibrils of the cell.

and extend to the centrum, where they enter the ganglion cells—usually through the main process of the cell, although fine fibrils may enter at other points—and break up within the somatoplasm into a fine network, the form and position of which varies in different types of cells. No fibril passes through a cell without branching and forming part of the network which surrounds the nucleus, but never penetrates it or unites with any of its elements. The fibrils do not remain in the cell, but unite to form a thicker primitive fibril, which may enter other ganglion cells and form a network in them, and then pass on to a muscle. In this way there is direct continuity between cellulipetal and cellulifugal fibrils.

Peripherally, the nerve breaks up before it reaches the sensory cells, and sends one primitive fibril to each cell (at least, in the worms and the molluscs). Inside the sense cell, a plexus is formed around the nucleus, after which the branches unite in a single fibril, which may pass out to the cuticle, or may take an intercellular course in the sub-cuticula.

A distinction is made between nerve and ganglion cells. The nerve cell is structurally the analogy of the muscle cell, and produces conducting substance just as the muscle cell produces contractile substance. The conducting substance consists, in the main, of the conducting primitive fibrils, just as the contractile substance consists of contractile fibrils. Ganglion cells are, to a certain degree, intercalated in the conducting nerve tracts, as current-generating elements of the electric battery are inserted in the conducting path of telegraph wires.

Ganglion cells produce that which shall be conducted; nerve cells, that which shall conduct.

At some stage in its development, a cell may begin to form a definite product, and through this differentiate itself so that it may no longer be demonstrable as a morphological unit, while it still forms a specific product which functions for it, and may have a nucleus and a more or less clearly differentiated cell body. With this explanation, he describes the nerve cell as having a nucleus lying within the lumen of the nerve fiber among the primitive fibrils, and separated from them by a spindle-shaped space. Near the nucleus the cell divides proximad and distad into branches which form nerve fibers. True anastomoses occur between the nerve cells by means of these branches.

In their origin nerve fibers may be considered more in the nature of protoplasmic cell-bridges which exist from the first division of the egg cell to the cells of the developed organism. All these are primarily merely intercellular bridges of undifferentiated protoplasm, whether they originate in this way or as processes of certain cells. Such a bridge may possess a certain power of conductivity, but it first becomes a nerve when the specific conducting substance, the neurofibril, is differentiated in it, just as a muscle cell, although it may be capable of contractility, becomes a muscle only when the specific contractile substance, the myofibril, forms, and is disposed in a certain way. No plexus is formed in the ciliated cells. Instead, a fibril enters at the base of the cell and divides into a brush, or pencil, one fibril of which goes to each cilium. The nerve fibril is distinguished from the cilium by its different reaction to stains, and by its appearance in polarized light.

In the muscles, one fibril penetrates each muscle cell, where it forks, without forming a plexus, or becoming intimately associated with the nucleus, and passes out of the cell. He finds a closed plexus extending through the epithelium of the nephridia, in the intestinal wall, and in the walls of the capillaries. There is a continuous going over of primitive fibrils from one conducting tract to another, both in the centrum and in the periphery, and there are anastomoses between the ganglion cells.

Knowledge of the extent of these nerve fibrils is limited by the power of the microscope. Apáthy is not able to find any definite ending for the finest sensory fibrils, and suggests that they may form a closed elementary plexus in the subcuticula, comparable with the capillaries of the circulatory system. "*Eine endigung des Leitenden ist nirgends zu constatiren.*"

E. M. B.

**Bernstein, J.** Zur Konstitution und Reizleitung der lebenden Substanz. Biol. Ctrbl. 19: 289-295, 1899.

(1) The irritable living substance of nerve and muscle fibers is composed of molecules imbedded in nutrient fluid—paraplasm, neuromusculum, sarcoplasm, cell sap. Molecules are aggregated in longitudinal rows in fibers or fibrillæ, and held together by chemical affinity which is equivalent to chemical valence.

(2) The rows of molecules may be polarized longitudinally, but not transversely, by the electric current.

(3) It is assumed that there are certain atomic groups adhering to the

molecules which may be thrown off through changes due to activity, and renewed from the nutritive fluid (restitution, assimilation). Stimulation causes separation of these atoms.

(4) At the cathod, ions unite with the atomic groups laid there and produce action by augmentation.

The molecular rows of the axis cylinders and of the contractile substances may be separated out in the differentiation of nerve and muscle substance, from the protoplasm, in the same way in which we may suppose that molecular rings are formed into the thread-like structures of the nucleus during the phylogenetic changes of the living protoplasm.

E. M. B.

## NEWS AND NOTES.

WOOD'S HOLL MARINE BIOLOGICAL LABORATORY.—The twelfth session of the Marine Biological Laboratory at Wood's Holl, Massachusetts, opened June first. The regular work of different classes will begin in the latter part of June, and the first of July. Instruction will be given by specialists in embryology, cytology, animal morphology, general and comparative physiology, comparative psychology, and botany. There will be day and evening lectures by leading investigators of this country, and seminars for the discussion of recent progress in different branches of biology. Applications for tables should be made to the director, Dr. C. O. Whitman of the University of Chicago, or to Dr. T. H. Montgomery, jr., University of Pennsylvania.

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COLD SPRING HARBOR MARINE BIOLOGICAL LABORATORY.—The regular class work of the Marine Biological Laboratory at Cold Spring Harbor, Long Island, will begin July 5th and continue for six weeks, although special students may use the laboratory from the middle of June until the middle of September, if desired. The director, Dr. C. B. Davenport, of Harvard University, will be assisted by specialists from various universities, and there will be a course of evening lectures by prominent scientists. The laboratory has a pleasant location on the northern coast of Long Island, near the New York Fish Commission, and is only about thirty miles from New York. While every facility is offered for investigation, teachers and others who wish instruction in new lines of work will find courses well adapted to their wants, and will also have opportunities to get in touch with important questions of the biological world. Information regarding the school may be obtained from Prof. Franklin W. Hooper, 502 Fulton street, Brooklyn, N. Y.

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THE AMERICAN MICROSCOPICAL SOCIETY.—The twenty-second annual meeting of the American Microscopical Society will be held at Columbus, Ohio, August 17, 18, and 19, 1899. Reduced rates of a fare and a third have been secured, and a large gathering of microscopical workers is expected. The meetings will be held in the elegant new Biological hall of the Ohio State University, which was designed for the special accommodation of the departments of entomology, zoölogy, anatomy, and physiology, and is fully equipped with all the



latest and best apparatus for this work. The building is, in design and general appearance, one of the most attractive on the campus, and is easily accessible from the city hotels. All the facilities of the institution will be placed at the command of the society, which is thus insured the most satisfactory environment for a successful meeting.

The programme will contain the contributions of some of the most active and influential workers in the microscopical sciences, and will compare favorably with the scientific work accomplished at previous meetings.

A portion of the programme has been arranged especially for teachers of the natural sciences in our grammar and high schools, and, with this end in view, Professors S. H. Gage, and V. A. Moore of Cornell University, Dr. A. C. Mercer of Syracuse, the president, Dr. Wm. C. Krauss of Buffalo, the secretary, Professor H. B. Ward of Lincoln, Neb., and others, will present papers dealing with technique and laboratory methods best suited for high school teachers and private workers. In addition to this, papers embodying the results of the most advanced investigations have been promised by original workers in the several departments of biological science, which will be of value and interest to the more advanced workers with the microscope.

Thus, with reduced rates, an admirable meeting place, a hearty welcome, an unexcelled programme, both for the elementary and advanced worker, and the cordial support of the officers of the society, the Columbus meeting promises to be one of the best the society has ever held. Every member of the society should, if possible, be in Columbus August 17, 18, and 19, 1899. Titles of papers may be sent to the president or secretary.

HENRY B. WARD, Secretary,  
Lincoln, Neb.

WM. C. KRAUSS, M. D., President,  
371 Delaware Ave., Buffalo, N. Y.

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AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE.—Columbus, Ohio, where the next meeting of the American Association for the Advancement of Science is to be held, was for many years the home of William S. Sullivant and Leo Lesquereux. It is now more than a quarter of a century since the death of Sullivant, the greatest of American bryologists. It is, therefore, intended to set apart a day for a bryological memorial meeting. The committee which has the matter in charge is planning a series of historical papers presenting the bryological work of the great masters in this subject, to be illustrated by type specimens, books, presentation copies of pamphlets, autograph letters, etc. European bryologists who have worked upon North American mosses will be asked to contribute. We shall hope to publish further details when the plans are further developed.

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When preparing earthworms for sectioning, particles of sand and gravel in the alimentary canal, that might injure the microtome knife when the specimen is cut, may be removed by feeding the worms bits of filter paper before killing.

In laboratories that do not have running water, a source of supply may be improvised by using any large cask that is fitted with a spigot. The cask may be placed on a stand to raise it to a convenient height, and filled with water as often as required.

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DETROIT CENTRAL HIGH SCHOOL.

## The Biology Work in the Detroit Central High School.

The school occupies the entire half of a block and is therefore not in the shadow of any other building. It faces slightly south of east. The three laboratory rooms for biology are on the second floor and have west and south, north and west, and north and east exposures, the facings thus given being the longer sides of the rooms, lighted in each case by five windows, and the shorter by three or four. The window seats are forty-eight inches from the floor, a little higher than is usually considered best for laboratory purposes, but the height of the windows, reaching within fifteen inches of the ceiling, compensates for this, and the long tables are evenly lighted from one end to the other. Those windows

on which the sun strikes are provided with white roller-shades in addition to the brown ones furnishing all the other windows. These permit an agreeable light in spite of the sun, and on dark days our electric light plant renders work possible.

Although one of the rooms is at the opposite end of the building from the other two, communication is easy and quick between these and other rooms and the office by a system of auto-telephones. In addition to three stairways, two elevators carry students to and from the second floor.

The larger botany room has a conservatory adjoining in which plants are kept for experiment and observation, but each room has a supply of plants in the windows and on tables—collected mostly by the teachers. There is gas and water



ZOOLOGY ROOM.

connection in each room, with especial fittings in the zoölogy laboratory for aquaria, and a set of basins, in this room and in one of the botany rooms, furnished with hot and cold water.

During school hours—8:30 to 1:10—fresh air and heat are supplied by a system of blowers, and at other times the rooms are heated by steam. On account of the plants, and germination and seedling experiments carried on during the cold weather, the steam heat is important. Yet it is not impossible to do this work without.

The ceilings are white and the walls gray, reflecting an agreeable light. The latter are broken below by slate blackboards that entirely surround all rooms, and in no well regulated laboratory are they idle. The rooms measure, respec-



tively, 51 feet 7 inches, by 29 feet 3 inches; 51 feet 7 inches, by 27 feet 4 inches; 48 feet 8 inches, by 24 feet 9 inches. From floor to ceiling there is a depth of 16 feet 5 inches. It is partly the height of the rooms and windows, and the arrangement of the latter that causes visitors to remark, "How pleasant your rooms seem." There are no special recitation rooms, teachers' rooms, or store-rooms.

The tables differ somewhat in each room and may be briefly described as follows. In one of the botany rooms the four student tables are of heavy oak, 30¾ inches high, 145 inches long, 72 inches at the wider end turned towards the windows, and 45 inches at the opposite end. They are, in other words, flat-iron-shaped and are supposed to give each student farther from the source of



L. MURBACH, PHOTO.

LARGER BOTANY ROOM (WITH CONSERVATORY).

light, more than he would otherwise get; but in well lighted rooms this is of little practical importance. There are a cabinet end and eight vertical tiers of drawers with combination locks under each table top, for students' apparatus, books, paper, etc. The four drawers in each tier accommodate as many sets of students, corresponding to our four laboratory periods in biology. The working capacity of the room is thus seen to be thirty-two students; yet I think no laboratory teacher should generally have more than twenty-five. Ball-bearing rollers make it possible for one janitor to do the necessary shifting of tables.

In the other botany room the tables are oblong, 144 inches long, 45½ inches wide, and 31 inches high. They are without cabinet ends, but otherwise fitted with eight lockers like those above described. In the zoölogy laboratory the five



tables are also oblong, but have six tiers of four drawers each, and cabinet ends. They measure 102 inches in length, 46 inches in width, and are  $30\frac{1}{2}$  inches in height. Here the seating capacity is six students, instead of eight as in the other rooms. The differences in tables is of no importance and is due to their being planned at different times and by different teachers. All these tables answer well the two chief requisites for laboratory use—stability and plenty of room. Furthermore, large tables seating six or eight students save floor space, and are more conducive to quiet work than many small or individual tables such as are used in colleges. Each room has one or more supply tables, differing from the student tables principally in being higher (35 inches) and having fewer lockers. There is also a set



A CORNER IN THE CONSERVATORY.

of tables of this height for the practical work in physiology, at which students may work standing, or on stools.

The chairs are the revolving screw-top kind with rocking spring and back. On account of the different ages and varying stature of high school students, the adjustable chairs are important. They can also easily turn toward the teacher and rise without dragging back the chair or jarring the table, and a class exercise may take place at the laboratory tables, avoiding the confusion and loss of time incident to moving from a recitation room. In fact, no laboratory period should be allowed to pass without some general questions or explanations covering points found difficult by a majority of the class. It has a stimulating effect.

A large case for books, apparatus, and specimens, with glass doors and cabi-

net bottom, a wardrobe case (used in our laboratories mostly for apparatus and reagents), teacher's desk and chair, complete the furniture for the three rooms. Besides these there is in the zoölogy room a slated (chemical proof) four-compartment sink, with hot and cold water faucets, five basins also with hot and cold water, a double-deck aquarium table with four large plate-glass aquaria below and three smaller ones above, all supplied with running water for breeding and keeping animals. In each corner of the conservatory there is a basin with hot and cold water, and in the center an elliptical slate-top table hewn hollow, one and one-half inches deep. This serves well for transplanting or for irrigating a large number of potted plants at the same time. One of the two faucets in the center



SMALLER BOTANY ROOM.

may be converted into a spraying fountain. The floor of the conservatory is cement and may be flooded without harm. In two main features it is lacking—there is no glass in the roof, and no automatic heat regulation.

Each botany room has a plate glass aquarium. One of these illustrates an interesting experiment in heliotropism. A tin stencil of our D. H. S. monogram was placed on the side most strongly illuminated, and in three weeks the algae accumulated in this pattern, forming the letters in deep green. It has lasted over six months.

We have a typewriter for manifolding our laboratory directions, reviews, and examination questions. The new terminology is usually quite enough for the student beginning any study not to vex him with reading it in an unfamiliar hand.

Unless the classes are very small and the blackboard directly in front, the practice of writing directions or questions on this is open to serious objections.

Some of the principal apparatus may be enumerated to give an idea of what is useful. There are 30 BB. No. 4 compound microscopes, with double nosepiece,  $\frac{2}{3}$  and  $\frac{1}{6}$  inch objectives, and two oculars each; seventy-five Barnes' dissecting microscopes, a small microtome, paraffin bath, and besides these the usual miscellaneous outfit found in a biological laboratory. Scissors, forceps, and the necessary reagent bottles are furnished on each table.

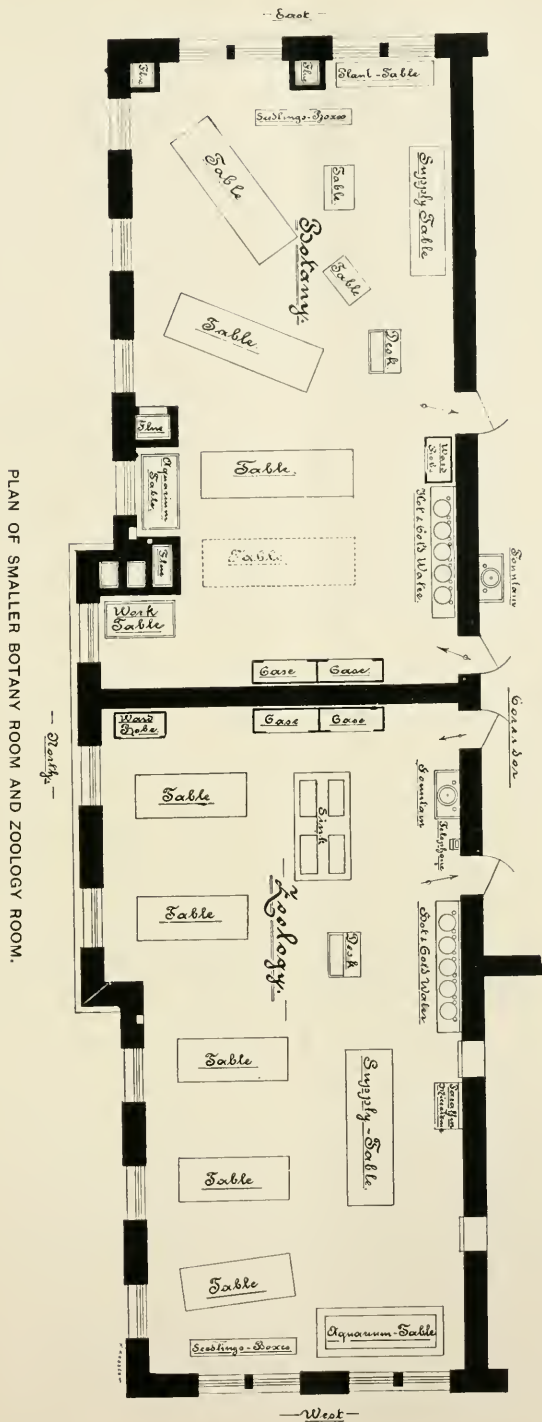
Each room has more or less its own particular outfit, depending on the work done in it. Thus, the compound microscopes, water bath, and microtome were originally purchased for the zoölogy, but may be used in the other work. There is also a set of preserved specimens illustrating the types from the sponges up. A complete set of reagents makes it possible to prepare permanent mounts of plant and animal tissues. For the work in botany there are bell jars, seedling boxes, candy jars for storing seeds, dishes of various kinds useful in germination experiments—a set of galvanized iron trays fitting the window seats, for potted plants, but also used for germinating seeds on the tables, when this work is in progress.

At present the work in physiology is done in the larger botany room. The principal aids are: a skeleton, two sets of anatomical charts, bone forceps, saw, chemical thermometer, lactometer, etc., and the necessary reagents for working out the food-stuffs in many common foods.

While the museum specimens answer well for illustrative material in zoölogy, student contributions are encouraged both in this and the other studies, for the reason that it awakens interest and pride in such a collection and valuable things are often brought to light. Outside a set of Hough's American woods, our collection for botany consists of woods, nuts, fruits, etc., given by students. This year a collection of pressed, dried plants representing the local flora has been begun.

In connection with the above may be mentioned our charts (over forty-five accumulated within three years) illustrating subjects studied in our courses. They are entirely the work of students and are not only as clear and as good as commercial ones, but frequently show artistic ability. Some of them are copied from books, but most of them are from nature. For such work we select students who draw readily and thus get that part of their work done sooner than the rest and spend the spare time on making a chart. So far, this kind of work has been done on heavy draughtsman's manila paper with charcoal, crayon, pencil, or brush, as the student chose.

It is aside from our purpose to plead with those who do not believe in the value of studying things themselves rather than learning many interesting facts about them from books. They will reach this conviction in the same way that they learn their daily lessons—after costly experience. We wish rather to help such teachers as are converted to the better (laboratory) method, but lack experience and means or ways. Of course some instruments are useful at the outset, and under the successful teacher's guidance the work will grow, creating a demand and the need for more and better facilities. A teacher tells me he can-





not do laboratory work in biology because he has no laboratory, no microscopes or other appliances. This is his excuse for using only a text-book. A school-room with plain desks, when these are used for dissecting and observing specimens, becomes a laboratory, and careful, patient examination with a good hand lens goes a long way toward microscopes. The work at present done in our course in botany would require only a hand lens (tripod), needles, and a knife, with such other things as can be brought by the students. A teacher who can work up a course from such beginnings will have no trouble in getting from school authorities the more convenient tables, microscopes, aquaria, microtome, and other apparatus. In other words, first start the course, and if well founded its demands will become apparent and imperative.

What has been said about apparatus applies in a way to the number of subjects or types covered in the course. It is not necessary, in fact not even desirable, to attempt covering the whole plant or animal kingdom in the time devoted to these subjects in the high school. This becomes patent when we reflect that it is now generally admitted that one of the chief aims of secondary education is training rather than informing. It is higher ability in a student to be able to reproduce facts than to memorize them. Studies for disciplining the mind, not didactic or informational ones, belong in the early education of children to prepare them for life in its broader sense—the finding of one's true place in nature and filling it in manly fashion.

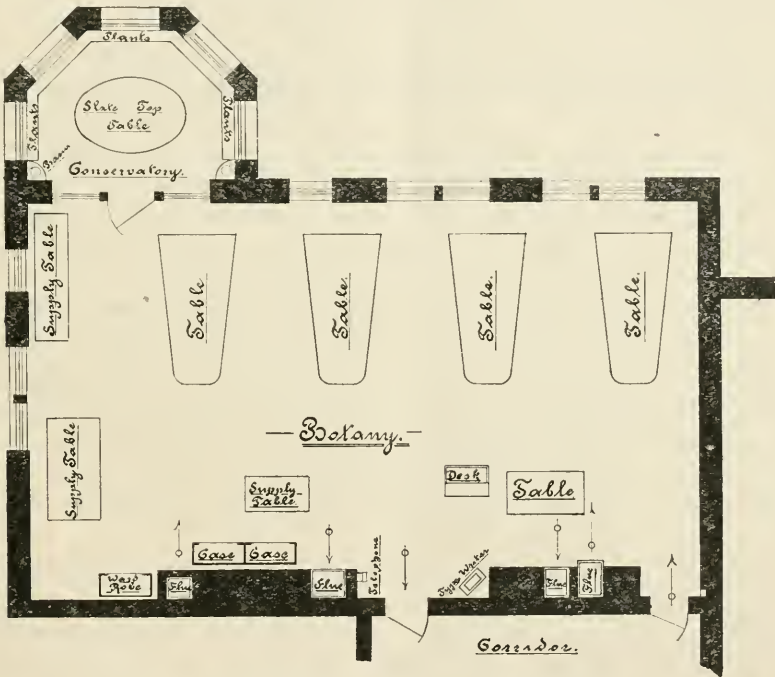
Above all things, laboratory work should teach thoughtful, painstaking and accurate observation of natural phenomena (facts), for unless this is done with method and surety, all subsequent inferences may be false, and so much valuable time is lost in a double sense. Too frequently only the more striking or interesting phenomena are brought out and the teacher becomes a showman instead of a true pedagogue. Next comes, naturally, methodical recording and grouping of facts that they may be compared with each other. Here it is important to allow time enough for giving its place to each fact according to importance, that the relevant facts may be at once recognized for use in the following step, which, though the most difficult, when thus thoroughly approached is now clear sailing—the drawing of conclusions. Then the formulating of principles and their clear record in drawing and writing are the lasting results of such a piece of work.

With young students it is necessary to make brief, comprehensive statements, drawing more rigid lines than exist in nature, for in their cases numerous exceptions and modifications lead to uncertainty and confusion. Begin anywhere in nature and study anything so long as you proceed logically and systematically from your starting point. As far as possible make out your own directions and questions to cover the exact ground, so that you do not have to tell the students "we must omit this" or "fill in that." Or if you must use a book (laboratory guide) have the best, and stick to any part you wish to use, rather omitting a whole subject than part of any one.

The trouble with most books of this kind is that they tell too much, leaving the student little chance for originality. He mechanically verifies what the book says, fitting his plant or animal to the description—rather than making his description from the specimen. Writing directions and questions on the board

causes much discomfort, distraction, and loss of time to the student. This should not be necessary when duplicating apparatus can be had or made for a few dollars.

We give frequent sets of review questions, allowing the student ample time for working them out and formulating his answer. This does not take the place of recitations, but supplements them, and is found helpful. The value of questions in training is brought out by the best of all older teaching. Have not most investigators been stimulated and led to the problem solved or the principles given to the world by their questioning spirit, aroused by keener observation than other men possess? If we had much more time for recitations in which—by appropriate questions—to help the student to a systematic handling of his obser-



PLAN OF LARGER BOTANY ROOM, WITH CONSERVATORY.

vations, the logical arrangement of his thoughts and clear expressions of his conclusions, the laboratory work might be given much greater value and permanence. Frequent written review questions do a great deal toward this.

In the high school curriculum (and also in primary work) biology should be placed earlier than physical sciences because the phenomena of living things appeal more strongly to the untutored mind and the principles can be stated in simpler and more informal manner, and are easier of comprehension. For similar reasons botany should be placed before zoölogy. On the other hand, physiology should follow zoölogy because it needs to draw on the knowledge of the animal body gained in the latter study. If students could be required to take all

three subjects instead of one, we should have a fair quota of time (one and one-half years) for our sciences. At present botany is the only subject required in all courses (fitting a requirement of the University of Michigan), giving students only one semester of laboratory work. The zoölogy and physiology are elective and are taken by a smaller number.

The biological studies regularly come in the second year of the high school course. The number of students varies from 300 to 350, divided among three teachers. Botany and zoölogy are given as full courses each semester, i. e., five times per week. Physiology extends over two semesters as a half course. The six recitation periods of the school (forty-two minutes each) are divided for biology in such a way that each student has two periods one day and one the next. For this single-hour day he may be required to prepare a lesson at home, otherwise all the work is done at school. Recitations occupy about one-fifth to one-fourth the whole time. It will be seen, then, that the work is mostly laboratory, and these lessons are always begun by placing in the hands of each student a set of directing questions and the specimens upon which they bear. This is the work for several days, or, until the subject is completed, with notes and drawings made at the time of observation. It is not good practice to take only scrappy and hurried notes from which to write up a complete account outside the laboratory. Memory, imagination, and the text-book are too often drawn on in such case and the work has not the same value. Generally, when a subject is finished the work is reviewed, the facts correlated, and general conclusions stated.

In botany the first semester is begun with flowers because then they may be had in abundance. The fruits follow as the ripened parts of flowers, then seeds, seedlings, roots, stems, buds and branches, and leaves, thus returning to the flowering stage of the plant. Before closing, the work is reviewed in the shape of the life-history of the flowering plant, which can be made to include all that the student has learned. This is rather more than can be profitably done and we contemplate cutting down the number of subjects, and perhaps introducing more experiments, especially such as illustrate the activities of plants.

We find experiments the most helpful way of thoroughly impressing facts on the mind. But in order to get their full value they should be performed by the students themselves as far as possible. Out of thirty-two students given a supplementary examination, twenty-five correctly explained an experiment illustrating respiration in plants. This and similar experiences certainly indicate the value of experiments. We give most of the experiments suggested by Bergen's *Elements of Botany*, though in a somewhat modified form.

The course in zoölogy begins with the lower forms covering as many types as can be thoroughly done, including a mammal if possible. One semester the experiment was tried beginning with higher forms first, but was not thought as satisfactory as the other way, which was again adopted. The laboratory work in each group is followed by a discussion of relationship. One period on Friday of each week is devoted to reports and discussion of such biological topics as can be understood by this grade of students. Once during each semester the class visits the Art Museum, where, after a lecture by the custodian, the zoölogical specimens are examined.

Physiology is in a transitional stage from text-book work, illustrated by apparatus and organs of animals dissected in class for the first half of the course, giving the student a fair idea of the skeletal and muscular systems, a more thorough knowledge of the alimentary, circulatory, respiratory, and excretory systems: then hygiene and sanitation, closing with what to do in case of emergencies. This is followed by a course devoted entirely to laboratory work on the gross structure and composition of bones, levers of the body illustrated, microscopic examination of muscles, tests for known food-stuffs, applied to find which of these are present in common foods, the changes taking place in germinating starchy seeds, a few digestion experiments, and one culture of common bacteria and yeast.

L. MURBACH.

Detroit, Mich., June, 1899.

## Preparing Sections of Cochlea for Microscopical Examination.

In preparing sections of the inner ear many difficulties arise which must be overcome before the work is at all satisfactory. The greatest difficulties we have found are those of orienting the material in the desired way, and keeping the material in its normal condition.

Having met with good success in obtaining sections of the inner ear, we here give our method of work.

We have found the best materials for the work to be the ears of young pups, and kittens, about two weeks old. We take the temporal bones from the animals as soon as the animals are dead, separate the petris bone from the rest, and place them in five per cent. nitric acid until they are thoroughly decalcified. The acid acts both as a killing and a fixing agent, and removes the mineral matter from the bone.

We have used the celloidin and paraffin methods extensively. We find the paraffin method by far the best, as it preserves the material in its normal condition. The celloidin method is good for general characters, but the shrinkage which must necessarily result when the celloidin has hardened, causes the membrane of Reissner, and the basilar membrane to be folded.

We have used many stains, and have obtained the best results with borax carmine in toto, and picric acid on the slide. The acid is especially good to bring out the hairs of the hair cells. We here give in detail the steps taken in the work. When the material is thoroughly decalcified place it in:

- (a) Rinse water for eight hours.
- (b) 35 per cent. alcohol, three hours.
- (c) 50 per cent. alcohol, six hours.

(d) Now take the material from the alcohol, and with a sharp razor make free-hand sections of the material in the direction desired. If the desired view is a longitudinal one, cut thin slices parallel to the auditory nerve and the modialis. This is easily done, as the spiral of the cochlea can be plainly seen



from the side opposite the entrance of the auditory nerve. Continue sectioning until the scala media is exposed from apex to base of cochlea.

- (e) 70 per cent. alcohol for four hours.
- (f) Borax carmine for twelve hours.
- (g) 70 per cent. acidulated alcohol until destained.
- (h) 85 per cent. alcohol, three hours.
- (i) 95 per cent. alcohol, three hours.
- (j) Place material in 2 oz. bottle containing about 2 inches of 95 per cent. alcohol; add about one-half inches of cedar oil, and allow the material to remain in this mixture until it sinks to the bottom of the bottle (do not shake the bottle).
- (k) Place the material in pure cedar oil for three hours.
- (l) Add to the pure oil, paraffin that has been dissolved in cedar oil; continue to add little by little until the oil will not dissolve any more paraffin; keep the bottle in the sun, or in some other warm place for a day or two, adding fresh paraffin for the first day.

(m) The material now being completely saturated with paraffin, place it in the paraffin bath for one hour, being careful to keep it just at the melting point. Now imbed, being careful to orient it so that the desired view may be obtained.

When the material has been sectioned, fix the sections, which need not be very thin, on the slides with Mayer's albumen, and allow them to dry. Then place the slides in:

- (a) Xylol (without melting the paraffin) for five minutes.
- (b) Absolute alcohol for two minutes.
- (c) 95 per cent. alcohol for two minutes.
- (d) 95 per cent. alcoholic solution of picric acid for one minute.
- (e) 95 per cent. alcohol two minutes.
- (f) Absolute alcohol two minutes.
- (g) Xylol five minutes.
- (h) Mount in balsam.

MEL T. COOK, Professor.

H. H. ZIMMERMANN, Assistant.

De Pauw University.

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A square of thin mica makes a good cover-glass to use in studying fish\*eggs, frog eggs, etc. On account of the flexibility of the mica, the egg is less liable to be crushed than with a glass cover, and if the effects of pressure on the development of the egg are to be studied, the mica cover is easily manipulated.

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A bottle for collecting insects may be made from an olive bottle, or any convenient wide-mouthed bottle. Place some potassium cyanide in the bottom, cover with plaster of paris, and sprinkle on enough water to wet the surface so that when it dries a hard crust will form over the top. Label *poison*. Keep the bottle closely corked, and be careful not to breathe the contents.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

## V.

## GENERAL REMARKS ON STAINING.

Many things may be examined alive without killing, fixing, staining, or any of those processes. A filament of *Spirogyra* shows the chromatophore nicely if merely mounted in a drop of water; the nucleus may be visible, and the pyrenoids can usually be located. Of course, such a study is necessary if one is to understand anything about the plant, and in an elementary class this might be sufficient, but a drop of iodine solution applied to the edge of the cover would emphasize certain details, *e. g.*, the starch in the pyrenoids would appear blue, the nucleus a light brown, and the cytoplasm a lighter brown. This illustrates at least one advantage to be gained by staining; it enables us to see structures which would otherwise be invisible, or almost invisible.

With so many stains at our disposal, it at once becomes a problem just which stain or combination to use in each particular case. Beautiful and instructive preparations occasionally result from some happy chance, but uniform success demands skill and judgment in manipulation, and also a knowledge of the structures which are to be differentiated. Let us take a vascular bundle for illustration. Safranin stains the xylem a bright red, but, with judicious washing, is entirely removed from the cambium and cellulose elements of the phloëm. A careful staining with Delafield's hæmatoxylin now gives a rich purple color to the cellulose elements which were left unstained by the safranin, thus contrasting sharply with the lignified elements. If cyanin and erythrosin be used, the xylem takes the blue and the cambium and phloëm take the red. Many terms have been given to indicate the affinity of certain tissues for certain stains. Auerbach used the terms erythrophilous and cyanophilous in 1890. This eminent zoölogist studied spermatozoa and ova. He found that if preparations containing both spermatozoa and ova were stained with cyanin and erythrosin, the nuclei of the spermatozoa took the cyanin, while the nuclei of the ova preferred the erythrosin; hence the terms cyanophilous and erythrophilous. Auerbach regarded these differences as an indication of sexual differences in the cells.

Rosen (1892) supported this theory, and even went so far as to regard the tube nucleus of the pollen grain as female, on account of its erythrophilous staining. In connection with this theory it was suggested that the ordinary vegetative nuclei are hermaphrodite, and that in the formation of a female germ nucleus the male elements are extruded, leaving only the erythrophilous female elements; and similarly, in the formation of a male nucleus, the female elements are extruded, leaving only the cyanophilous male elements.

As long ago as 1884, Strasburger discovered that with a mixture of fuchsin and iodine green the generative nucleus of a pollen grain stains green, while the tube nucleus stains red. In 1892 (*Verhalten des Pollens*), he discussed quite

thoroughly the staining reactions of the nuclei. The nuclei of the small prothallial cells of Gymnosperm microspores are cyanophilous like the male generative nuclei. The nuclei of a nucellus surrounding an embryo-sac are also cyanophilous, while the nuclei of structures within the sac are erythrophilous. His conclusion is that the cyanophilous condition in both cases is due to poor nutrition, while the erythrophilous condition is due to abundant nutrition. A further fact in support of the theory is that the nuclei of the adventitious embryos which come from the nucellus of *Funkia ovata* are decidedly erythrophilous, while the nuclei of the nucellus to which they owe their food supply are cyanophilous.

In division stages, nuclei are cyanophilous, but from anaphase to resting stage, cytoplasm is taken into the nucleus, and the cyanophilous condition gradually changes to the erythrophilous.

An additional fact in favor of this theory is that in *Ephedra* the tube nucleus which has very little cytoplasm about it is cyanophilous. Strasburger claims that there is no essential difference between male and female generative nuclei, and subsequent observation has shown that within the oöspore the sex nuclei are alike in their reaction to stains.

Malfatti (1891) and Lilienfeld (1892-3) claim that these reactions are dependent upon the amount of nucleic acid present in the structures. During mitosis the chromosomes consist of nearly pure nucleic acid and are intensely cyanophilous, but the protoplasm, which has little or no nucleic acid, is erythrophilous. There is a gradual transition from the cyanophilous condition to the erythrophilous, and *vice versa*, the acid structures taking basic stains and basic structures the acid stains.

The terms erythrophilous and cyanophilous are falling into disuse since the affinity is for basic and acid dyes, rather than for blue or red colors. That the terms are misnomers becomes evident when a combination like safranin (basic) and acid green (acid) is used, for the cyanophilous structures stain red, and the erythrophilous green.

Probably but few investigators who have attained any proficiency in microtechnique have not asked themselves how much dependence can be placed upon staining reactions as a means of analysis. Do staining reactions enable us to determine the chemical composition of a structure? If two structures stain alike with Delafield's hæmatoxylin, does this mean that they have the same chemical composition, or, if on the other hand they stain differently, must they necessarily be different in their chemical composition? Delafield's hæmatoxylin, when carefully used, gives a rich purple color, but a careful examination will often show that in the same preparation some structures stain purple, while others stain red. Does this mean that the purple and red structures must have a different chemical composition? Many people believe that structures which stain differently with a given stain must be chemically different, but they readily agree that structures which stain alike are not necessarily similar in chemical composition. Chromosomes of dividing nuclei and lignified cell walls stain alike with safranin; chromosomes and cellulose cell walls stain much alike with Delafield's hæmatoxylin, but every one recognizes that the chromosome is very

different in its chemical composition from either the cellulose or the lignified wall.

According to Fischer (1897), stains indicate physical but not chemical composition. Fischer experimented with substances of known chemical composition. Egg albumin was shaken until small granules were secured. These were fixed with the usual fixing agents, and then stained with Delafield's hæmatoxylin. The extremely small granules stained red, while the larger ones became purple. Since the granules are all alike in chemical composition, Fischer concluded that the difference in staining must be due to physical differences. With safranin, followed by gentian-violet, the larger granules stain red and the smaller violet; if, however, the gentian-violet be used first, then treated with acid alcohol and followed by safranin, the larger granules take the red and the smaller the gentian-violet. In root tips similar results were obtained. Safranin followed by gentian-violet stained chromosomes red, and spindle fibers violet, while gentian-violet followed by safranin, stained the chromosomes violet, and the spindle red. One often reads that chromosomes owe their strong staining capacity to nuclein, and especially to the phosphorus, but, according to Fischer, this is shown to be unfounded, since albumin gives similar results and yet contains no phosphorus, and is not chemically allied to nuclein. Delafield's hæmatoxylin is one of the so-called nuclear stains. The nuclei of animals and plants stain deeply with this reagent, but cellulose membranes, the dense protoplasm of embryonic cells, the pyrenoids of green algæ, and many other structures resemble nuclei in their staining. The most critical work on this subject has been done by those who are investigating the structure of the Cyanophyceæ and Bacteria, to determine whether these forms have nuclei or not. Bütschli claims that the granules which stain red with hæmatoxylin are to be identified with chromatin, while Fischer, whose results have just been given, claims that staining indicates merely physical differences. The subject can not yet be regarded as settled, but whatever may be true in regard to these conflicting theories, all agree that stains are of the highest importance in differentiating structures, and in bringing out details which would otherwise be invisible.

#### PRACTICAL HINTS ON STAINING.

In later papers specific directions will be given for making a series of preparations ranging from the lowest algæ to the flowering plants, but a few suggestions will be made here.

The number of stains in the catalogues is becoming so great that it is impossible to become proficient in the use of all of them. It is far better to master a few of the most valuable stains than to do indifferent work with many. The beginner, especially if rather unacquainted with the details of plant structure, may believe that he has an excellent preparation when it is really a bad, or at most an indifferent, one. To illustrate, let us suppose that a pollen-mother-cell in the spirem stage has been stained with cyanin and erythrosin. A preparation in which the cell merely shows a differentiation into nucleus and cytoplasm, must be classed as bad; if the nucleus shows a definitely outlined spirem thread, the preparation is better, but is still only indifferent; if the



thread appears as a delicate red ribbon bordered by blue granules, the staining may be regarded as a success. If mitotic figures have been stained with cyanin and erythrosin, a first-class preparation should show blue chromosomes and red spindles; if stained with safranin and gentian-violet, the chromosomes should be red and the spindles violet.

In staining growing points, apical cells, young embryos, antheridia, archegonia, and many such things, the cell walls are the principal things to be differentiated if the preparations are for morphological study. As a rule it is better in such cases not to use double staining, but to select a stain which stains the cell walls deeply without obscuring them by staining starch, chlorophyll, and other cell contents. For example, try the growing point of *Equisetum*. The protoplasm of such growing points is very dense. If Delafield's hæmatoxylin and erythrosin be used, the hæmatoxylin will stain the walls and nuclei, and will slightly affect the other cell contents, but the erythrosin will give the cytoplasm such a dense stain that the cell walls will be seriously obscured. It would be better to use hæmatoxylin alone. The same suggestion may well be observed in tracing the development of antheridia, archegonia, embryos, and similar structures.

Permanent preparations are an absolute necessity for the greater part of most advanced work, but let us not imagine that we cannot examine anything until we have made a permanent mount. It would be impossible to make a permanent mount of the rotation of protoplasm. It is better for many purposes to look at motile spores while they are moving. Use *Spirogyra* while it is fresh and green (if you can), and use permanent preparations only to bring out nuclei and other details, which are not so easily seen in living material. Examples might be multiplied.

*(To be Continued.)*

## The Demonstration of Alcohol and CO<sub>2</sub> in Yeast Cultures.

In laboratory work on yeast, the formation of alcohol as a result of the growth of the plant in a sugar solution should be demonstrated. The method usually employed—that of distilling off the liquid and demonstrating its character by taste, odor, inflammability, etc., is too troublesome for the average student, and I have found the iodoform test for alcohol much more satisfactory. If a few drops of iodine solution be added to the tube in which the yeast is growing, and then enough KOH solution to destroy the color of the iodine, iodoform will be produced, and can be recognized by its characteristic odor. It is best to perform the experiment first with a little dilute alcohol, and then compare with this result that obtained after treating the sugar solution as above. The CO<sub>2</sub> given off by the liquid in which yeast is growing can be demonstrated most easily by suspending a drop of lime water in the test tube on the end of a glass rod. The cloudiness set up in the lime water can be demonstrated much more easily, and just as satisfactorily in this way, as by the more complicated devices usually employed.

A. L. TREADWELL.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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THE microscope has been the servant of science almost from the time it emerged from the hand of the inventor, but it is not long that its value as a practical instrument in industrial institutions has been recognized. In fact it is to-day employed to only a limited extent even in those industries in which it should be universally used.

To a bacteriologist it would seem incredible that, in the great canning establishments throughout the country, ton after ton of vegetables, fruits, meats, and soups should be daily sealed up

and stored away without a single culture or microscopical examination being made to test the efficiency of the sterilizing apparatus or the bacterial cleanliness of the process. As a matter of fact things go right as a rule, but when a hardy organism *does* persist in growing inside the sealed-up cans, it often costs thousands of dollars and weeks of time to discover and rectify what might have been certainly and easily avoided by the systematic employment of the ordinary bacterial methods. A botanist can hardly imagine how the starch merchant can adulterate wheat starch with a liberal quantity of corn starch, offer it for sale as pure, and expect to escape detection, when a moment's use of the microscope will show the presence of the adulterant as unmistakably as the touch distinguishes the presence of thorns among leaves, and yet thousands of steam laundries are using "Special" and "Pure Wheat" starches which contain a large proportion of corn starch, at or near the price of wheat starch, when they might be had at their true value were their constituents known to the purchaser. The micro-structure of steel and other metallic substances often tells more of their adaptability for a particular purpose than can be learned by physical tests, and tells it quicker, still there are few users of metals who know anything of the methods or apparatus required. The microscope is a valuable aid in the examination of textile substances and the fabrics made from them, of many minerals, earths, and chemicals used for pottery and building materials, and of paper, to determine structure and quality, of handwriting, to determine its genuineness and age, of drugs, food stuffs, abrasives, and powders, to determine their purity. In most of these instances more elaborate and less effective tests, if any, are used. What a boon to the industries a few of the facts so well known to scientific men would be were they converted from the abstract to the concrete. The biologist can not apply his knowledge to industries of whose needs he knows nothing, and the manufacturer, not being a biologist, knows nothing of the possibilities of science. When the demand for teachers has been satisfied our graduates in science will find golden fields awaiting them in the industrial application of the facts and laws of biology, in the making of manufacturing processes less complex and more certain, thus increasing the producing power of each worker and decreasing the cost of the article produced.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Schrenk, Hermann von.** A Sclerotoid Disease of Beech Roots. Contributions from the Shaw School of Botany, No. 13. Report Mo. Bot. Garden. 10: 61-70, pl. 55-56. 1899.

A large number of root tubercles attached to the fibrous roots of a clump of beech trees led to these investigations.

It has long been known that roots of the beech, when growing in humus soil, are surrounded at their tips by a mantle of fungus threads known as mycorrhiza. Such roots branch freely, are usually coralloid in form, and grayish white in color, but are not contorted or hypertrophied. This mycorrhiza form of root was found on all the beeches of the locality, but the tubercles described in this paper were confined to certain clumps of trees. The tubercles are grayish white bodies, varying in size from almost invisible specks to masses as large as small peas. The tubercles consist of a number of contorted, interwoven roots, covered by a fungus sheath in which two layers can be readily distinguished. The outer, thicker layer is composed of large septate hyphæ, which branch freely and anastomose, thus forming a dense sheath which can be pulled off. The inner layer is composed of very fine, interwoven hyphæ, which also surround the individual rootlets. The epidermal layer of the root has a peculiar structure, the cells being elongated so as to resemble a palisade. The hyphæ are closely appressed to these epidermal cells, but unlike the hyphæ in mycorrhiza, they do not enter. The root tip is simplified, the cap consisting of only two layers of cells. The meristem cells are larger than those in roots of the mycorrhiza. The fact that many small sclerotia were found associated with the tubercles suggests that the hyphæ may belong to some Hymenomycete. The fungus of the outer layer of the sheath is probably distinct from that forming mycorrhiza. The occurrence of several different forms of mycelium, some closely associated with roots and claiming symbiotic relationship, and others more distinct from the root, as in the case of the root tubercles, suggests that the relation of root fungi to trees is still an unsolved problem.

C. J. C.

**Campbell, D. H.** Notes on the Structure of the Embryo-sac in *Sparganium* and *Lysichiton*. Bot. Gaz. 27: 153-164, pl. 27, 1899.

The most striking feature in the embryo-sacs of both these forms is the remarkable development of the antipodal cells.

In *Sparganium* the number sometimes reaches one hundred and fifty, thus exceeding the extensive antipodal development of grasses. In *Lysichiton* the number of antipodals is not so large, probably never exceeding ten, but they increase considerably in size. In both forms this vigorous development begins after fertilization. While the antipodals probably represent prothallial tissue, they must not be regarded as mere vestiges of a prothallium, since their great development and relation to nutrition shows that they are also of physiological importance. The behavior of the antipodals, and also the morphology of the embryo

in these forms, may indicate relationship with the grasses. The embryo-sac of composites shows a similar antipodal development, but if the single terminal ovule of composites is primitive rather than secondary, the embryo-sac might also show primitive characters.

C. J. C.

**Going, Maud (E. M. Hardinge).** Field, Forest and Wayside Flowers, with Chapters on Grasses, Sedges, and Ferns. 8vo pp. XVI + 411, figs. 102. The Baker and Taylor Company, 5 and 7 E. 16th St., New York, 1899, \$1.50.

The book is designed for those who have not time or perhaps inclination to familiarize themselves with the technical terms of botany. Some of the principal topics discussed, are: Crocuses, Dandelions, Flowering of Forest Trees, Green Leaves at Work, Roses, Lilies, Grasses, Rushes, Sedges, Night Flowers, Climbing Plants, Ferns, Thistles, and Winter Woods. In spite of the fact that the book is for the untechnical, it seems an unnecessary teaching of incorrect relationships to speak of grasses and sedges, as in the title, as if they were not flowering plants, but a separate group like the ferns. The language is simple and pleasing, and the subject matter interesting. The half-tone illustrations taken from photographs are of unsurpassed excellence. The book can be recommended to teachers of nature study in the schools as well as to those for whom it was written.

C. J. C.

**Fischer, A.** Untersuchungen über den Bau der Cyanophyceen u. Bakterien. Jena: G. Fischer, 8vo, M. T., 1897.

It has long been a matter of dispute whether the Cyanophyceæ and Bacteria have nuclei or not. Fischer had already written upon the subject, but the present account is the most important contribution which has appeared. He admits that Bütschli's central body cannot be explained as contracted cell contents, as he had formerly claimed, but still insists that there is no such sharp distinction between central body and "Rindenschicht" as Bütschli's figures indicate. After many experiments in staining bodies of a known chemical composition, Fischer comes to the conclusion that staining reactions do not depend upon a chemical union between the stain and the tissue elements, but that the phenomenon is merely a physical one. He claims that Bütschli was mistaken in the assumption that digestion experiments support the theory that the central body is a nucleus. The green rind of the cyanophyceous cell is a genuine chromatophore, and can be isolated by reagents. While he was not able to demonstrate a layer of protoplasm between the chromatophore and the cell wall, he nevertheless believes that plasmolytic phenomena and the collecting of granules along the cross walls indicate the presence of such a layer. The ground mass of the central body is nothing but the principal part of the protoplast which is surrounded by the chromatophore. This ground mass does not take any part as an independent organ, either in cell division or in spore formation, nor do the granules play any characteristic part during division. In short, there is no nucleus or organ which resembles a nucleus in the Cyanophyceæ nor is the central body a phylogenetic forerunner of the nucleus.

In the sulphur bacteria the central body was found only in forms which are free from sulphur. There are granules in *Chromatium* and *Beggiatoa* which stain red with hæmatoxylin, but they cannot be called chromatin on such evidence. There are no genuine nuclei in the sulphur bacteria.



In considering the genuine bacteria, he regards the theory that bacteria are nuclei without cytoplasm as unfounded. In well fixed material neither *Spirillum* nor other bacteria show clear, less deeply staining ends. The more deeply staining granules are not nuclei or chromatin granules, and present methods fail to demonstrate a nucleus. The relationship of the sulphur bacteria and all other bacteria to the Cyanophyceæ is merely one of loose, superficial morphology. They are more closely related to the Flagellatæ.

The technique throughout is thoroughly up to date. Microtome sections from paraffin material were stained in the most approved manner, and the plates are exceptionally elegant and definite in details.

C. J. C.

#### RECENT LITERATURE.

- Buscalioni, L.** Sopra un nuova caso di incapacamento dei granuli di amido. *Malpighia*, **13**: 3-13, pl. 1, 1899.
- Daniel, L.** La variation dans la greffe et l'hérédité des caractères acquis. (*fin.*) *Ann. des Sci. Nat. Bot. Ser. VIII*, **8**: 193-226, pl. 1-10, 1898.
- Halsted, B. D.** Root Tubercles and Nitrogen Appropriation. Extract from the 26th Ann. Rep. of the New Jersey State Board of Agriculture, Jan. 1899.
- Pee-Laby, F.** Étude anatomique de la feuille des Graminées de la France. *Ann. des Sci. Nat. Bot. Ser. VIII*, **8**: 227-347, pl. 11-13, 1898.
- Perrot, E.** Anatomie comparée des Gentianacées. *Ann. des Sci. Nat. Bot. Ser. VIII*, **7**: 105-202, pl. 1-9, and 29 figs. in text, 1899.
- Ricome, H.** Recherches expérimentale sur la symétrie des rameaux floraux. *Ann. des Sci. Nat. Bot. Ser. VIII*, **7**: 293-396, pl. 10-13 and 13 figs. in text, 1899.
- Schrenk, H. von.** A disease of *Taxodium* Known as Peckiness, also a Similar Disease of *Libocedrus decurrens*. Contributions from the Shaw School of Botany, No. 14. Report of the Mo. Bot. Garden, **II**: 1-55, pl. 1-6, 1899.
- Voglino, P.** Di un nuova malattia dell' *Azalea indica*. *Malpighia*, **13**: 73-86, pl. 2-3, 1899.

### ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
Agnes M. Claypole, Sage College,  
Ithaca, N. Y.

#### CURRENT LITERATURE.

- Carlier, E. W.** Note on the Ovarian Ova of the Hedgehog. *Journ. Anat. and Physiol.* **33**: 304-308, 1899.

These ova are remarkably compact in form and have peculiarly large nucleoli, and hence are favorable for the study of these features. The Graafian follicle is early represented as a layer of flattened cells arranged round the ovum; the nucleolus is strikingly large, about two microns in diameter, and taking a homogeneous stain. As the follicle cells become arranged in a double row, the nucleoli increase in size, and many "yolk" nuclei appear in close contact with the nuclei, but in the cytoplasm. These latter eventually migrate to the periphery and spread out into a layer of flattened bodies round the margin of the egg. The nucleus by this time shows irregular outlines and moves from its originally central position. The nucleolus now shows at least one vacuole and measures at least seven microns in diameter. This nucleolus may now be bodily extruded and another form, or vacuolation, may continue until a frothy, spongy mass results, taking the stain but slightly. The author concludes that the nucleolus is an accumulation of waste products that is eventually

disposed of, since it increases in size during the greatest growth periods, and is removed before mitosis. He does not consider it important as a nutritive or circulatory element.

A. M. C.

**Alcock, R.** The Peripheral Distribution of the Cranial Nerves of *Ammocetes*. *Journ. of Anat. and Phys.* **33**: N. S. 13, 131-153, 1 pl. 1898.

The author reviews the work of older anatomists on this question and takes as special points for study: 1. The innervation of the tentacles round the mouth. 2. The innervation of the epithelial pit sense organs on the head and bronchial region of the body. 3. The peripheral distribution of the facial nerve which has been said to be purely sensory. 4. The innervation of the thyroid and of the bronchial muscles. 5. The destination of the lateral line branch of the vagus and the *recurrens facialis*. 6. The limits of the vagus in the alimentary canal. 7. The existence of a sympathetic system. In this paper the distribution of the bronchial, facial, glosso-pharyngeal and vagus, and the arrangement and innervation of the sensory epithelial pits of the head and bronchial region of the body are alone considered, the remaining points being left for a future contribution.

The distribution of nerves was followed by means of serial sections stained variously but most successfully with picro-carmine. The nerves being all non-medullated made osmic acid useless for differentiation. Nigrosin, hæmatoxylin, borax-carmine, and osmic acid were all tried with varying results. Picro-carmine gives a deep red color to the outer sheath and nuclei, and a yellowish tinge to the axis cylinders of the fiber. Difficulties were introduced by the action of picro-carmine on the outer skin layer, prolonged immersion of the animal for several days at a temperature of 60 degrees C. caused the disappearance of this layer by solution, and too short an action made it become very hard and granular and difficult to cut. Motor and sensory fibers may be readily distinguished in spinal nerves by their difference in size, but in the cranial nerves all fibers are extremely small, and distinction by size is impossible.

Briefly summarizing the results, the author finds: That each bronchial nerve is entirely confined to its own segment as far as the motor and sensory nerves are concerned; there is no *ramus prætrematicus* nor *ramus posttrematicus*. Each bronchial nerve consists of motor nerves to the striated muscles of a segment; sensory nerves to the diaphragm, and dorsal and ventral nerves to the lateral line sense organs. The facial has all the components of a typical bronchial nerve and hence belongs to the glosso-pharyngeal and vagus group of cranial nerves and not to the trigeminal. The thyroid gland is innervated by a branch of the facial, and therefore belongs to the hyoid segment. The facial gives off a branch to all the tubular muscles in the bronchial segments. The sense organs of the lateral line system on the head and bronchial region are innervated by dorsal and ventral branches of the facial, the glosso-pharyngeal and the six bronchial divisions of the vagus. The lateral line sense organs posterior to the bronchial region of the body are innervated by the lateral line branch of the vagus.

A. M. C.

**Bolton, J. S.** On the Range of Applicability of Certain Modifications of the Weigert-Pal process. *Journ. of Anat. and Physiol.* **33**: N. S., 13; pp. 292-300, 5 pls., 1899.

The writer has previously published in the same periodical (**32**: 247-266,) a discussion on the application of the Weigert-Pal method as applied to formalin hardened tissue, and in the present paper treats of the relative merits of certain modifications and the range of their applicability. All material is hardened in five per cent. formalin and is prepared by the osmic acid, iron-alum, or ammonium-molybdate methods earlier described. In regard to the relative merits of the three methods the author says that for the coarser nervous tissue—for instance, human—osmic acid is invaluable, owing to its blackening the medullary sheaths. In finer tissue or with non-medullated fibers, as in the minnow, results are inferior. Iron alum with the author proves a most successful mordant. Axis cylinders and medullary sheaths are deeply stained, and especially fine fibrils, as in the case of small fishes, are well worked out; occasionally nerve cells are also finely stained. Sections require careful washing between the iron-alum and logwood bath, and the iron-alum solution should be freshly prepared and not used a second time. Ammonium molybdate is most satisfactory in its results, and with a yellow screen sections will photograph well; it is not as satisfactory for the finest fibers as iron alum, owing to its fainter color. The bath can be used repeatedly, however, but sections must be hastened through the alcohols. Osmic acid is the weakest mordant of the three, it is the least likely, with care, to stain bare axis cylinders or collaterals. Iron alum is the most powerful of the three, staining all the nervous network including nerve cells. The chief conclusion drawn by the author from his experience, is that the Weigert-Pal process is not a specific method for staining medullated fibers with hæmatoxylin, but dyes fibers exclusively after three steps, mordanting the fibers, forming a lake in them and finally removing the stain by oxidation from nearly all the other parts of the complex tissue. Only those parts of the tissue possessing a high degree of metabolic activity take the stains and react to the different steps, so a difference on results is readily explicable. Reference to the photomicrographs with which the article is illustrated shows that the methods are applicable to the human cerebral cortex, brain, cord, peripheral nerves and ganglia of mammals, and the nervous system of the frog and fish. Hence it is clear that the Weigert-Pal method is capable of wide application without much modification.

A. M. C.

**Krompecher, E.** Beiträge zur Lehre von den Plasmazellen. *Beitr. zur pathol. Anat. u. zur allegem. Pathol.* **24**: 163-182, 1 pl., 1898.

These studies were carried out on different kinds of pathological material. Alcohol was chiefly used for fixing and hardening, sometimes also sublimate. Sections were stained in methylen blue (polychromic) and thionin, and decolorized with alcohol or glycerine-ether according to Unna. Both methods were equally successful. Sections were stained in methylen blue from a quarter of an hour to over night, and after washing in water were brought direct into the glycerine mixture for about fifteen seconds, when differentiation is complete; washed carefully in water, and in absolute alcohol and bergamot oil, and mounted in balsam. For demonstrating the basophil granules the author used, besides the conventional polychromic methylen blue,

hardening, sometimes also sublimate. Sections were stained in methylen blue (polychromic) and thionin, and decolorized with alcohol or glycerine-ether according to Unna. Both methods were equally successful. Sections were stained in methylen blue from a quarter of an hour to over night, and after washing in water were brought direct into the glycerine mixture for about fifteen seconds, when differentiation is complete; washed carefully in water, and in absolute alcohol and bergamot oil, and mounted in balsam. For demonstrating the basophil granules the author used, besides the conventional polychromic methylen blue,

Winternitz's method for tubercle. Sections are stained in a two to three per cent. anilin water solution of fuchsin, decolorized in a 50 per cent. alcoholic fluorescein solution till the sections appear a light rose color, then they are counterstained with methylen blue. The basophilous granules are most clearly differentiated by their red color.

A. M. C.

**Tryaska-Chezonszczewsky.** Ueber meine Methode der physiologischen Injection der Blut und Lymphgefäße. Virchow's Arch. 153: No. 1, p. 110-129, pl. 1, 1898.

The author sets forth details of his old but very successful methods of physiological injection, dealing with the processes for different organs separately.

1. For the blood vessels of the spleen a neutral ammoniacal cochineal-carmine solution, 10 to 100 cc., according to the size of the animal, is injected into the jugular vein; after five minutes, before the carmine begins to appear in the urine, the abdominal cavity is opened and the veins, arteries, and the spleen cut out and hardened in 80 per cent. alcohol. After hardening microscopical preparations are made in the usual way and mounted in Canada balsam and Tammar shellac.
2. For the vessels of the liver a double physiological injection of veins and lymphatics was obtained by injecting indigo-carmine into the blood of the living animal and after ten minutes, before the coloring fluid appeared in the kidneys, cochineal-carmine is injected into the blood. After five minutes the abdominal cavity is quickly opened and the vessels of the liver ligatured. In sections prepared from this material blood vessels are stained with cochineal-carmine and round them lymph vessels full of lymph, and stained blue with indigo-carmine.
3. Lymph vessels of the lungs. The action of carmine toward living lung parenchyma differs greater from its action toward dead tissue. It stains neither intercellular substance nor the cells of the different tissues; it also leaves unchanged the epithelium of the bronchi with which it comes into undoubted contact. It brings out particularly the characteristics of lymph vessels. The carmine goes from the bronchi to the cells of the inner tissue, through the finest canals of the lung alveoli whose stomates lie between the epithelial cells. After a quarter of an hour the urine of the animal is colored red with carmine. The animal is then killed quickly with chloroform and the lungs injected with Berlin blue through the pulmonary artery. The lungs are then taken out and hardened in alcohol or cut frozen.
3. The lymph vessels of the diaphragm.—10 to 100 cc. of neutral ammoniacal cochineal-carmine solution is injected into the abdominal cavity, the amount varying with the size of the animal. After five to ten minutes, as soon as the urine shows color, the diaphragm is removed and washed in clear water and hardened in 70 per cent. alcohol; then cleared in turpentine or another ætherial oil, and mounted in Canada balsam or Tammar shellac. With the naked eye the injection of the large and medium sized lymphatics can be seen, and under the microscope the very finest.
5. The connective tissue between the blood and lymph capillaries.—An animal is injected with carmine solution through the external jugular vein, and after five minutes the abdominal cavity is opened. Cutting through the lower part of the diaphragm, the flap is turned back and quickly washed, first in pure water and later in a solution of silver nitrate. Then the diaphragm is cut out, put into 80 per cent. and then absolute alcohol, cleared,



and mounted. These preparations show clearly the blood vessels carmine stained, and filled with blood, while the large and small lymphatics are brought out by the silver nitrate. Along their whole course one sees clearly a rose carmine color which is neither in the ground tissue of the diaphragm nor in the connective tissue of the lymph spaces. These spaces are closely pressed into triangular forms in the region of the blood capillaries. The inside of these triangular enlargements of the spaces, as also the spaces themselves, are colored with the carmine, while the connective tissues remain unchanged. 6. The lymph vessels of the skin.—The skin is found to be pervious to aqueous solutions and the following method is used to demonstrate the course of the lymph vessels. Cochineal carmine is used, with this precaution, that there shall be an excess of carmine in the solution to prevent the maceration of the skin by the ammonia. The animal is put into this liquid and the steps of the color can be followed. After three hours, carmine has penetrated the epidermis, later the hair bulbs, the outlets of the sebaceous glands, and the glands themselves. After six hours the coloring has spread to the connective tissue, bringing out the spindle and stellate elements and their processes with great clearness. The lymph vessels form a thick net which rise in the papillary layer in the form of loops, lying below the blood capillary loops. This superficial network passes directly into the deeper and larger lymph vessels. To demonstrate the absorptive powers of the granulation layer, a longitudinal piece of skin was taken from the backs of dogs and other animals, the wound treated in the customary way. After the healing granulation tissue had formed, cochineal-carmine or indigo-carmine solution was dropped upon the wound. In vertical sections through the whole thickness the red or blue stained capillaries were most clearly visible.

A. M. C.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review  
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**Novy, Dr. F. G.** Laboratory Work in Bacteriology. University of Michigan. Pp. 563. George Wahr, Ann Arbor.

Laboratory manuals in bacteriology are becoming somewhat common, but Dr.

Novy is to be congratulated in having produced one which will certainly be among the most useful for bacteriological students. Although the title indicates that it is a book designed for laboratory work, it proves to be more than this, since it gives a brief course in general bacteriology. The first five chapters give a general account of bacteria, their structure, life history, and relations to the general problems of fermentation. This introduction is excellent, and admirably serves to inform the student in regard to the general nature of the organisms which the rest of the work gives him directions for studying in detail. In choosing the subjects to be treated in this brief introduction, Dr. Novy has been successful in selecting topics of real

importance. The treatment of the subjects is perhaps a little too dogmatical, and conclusions of doubtful accuracy are occasionally given with all the assurance of demonstrated fact. For example, it is stated twice as a positive fact that the great soda nitrate beds of Chili and Peru owe their origin entirely to the action of bacteria upon the excrement of birds. Now this may very likely be true, but Dr. Novy must certainly be aware that some scientists have doubted this conclusion and suggested that these deposits are to be traced to marine plants. Whether this suggestion be probable or not, the matter is hardly so surely proved as to merit the unqualified statement made by Dr. Novy.

Most of the book is, however, devoted to the methods of laboratory work. The descriptions of methods are clear and are accompanied by running comments in regard to the purposes of different details which decidedly enhance their value. These chapters are designed for the student in the laboratory to follow, and contain a description of some forty of the most important species of bacteria, both pathogenic and non-pathogenic, as well as an account of a few of the most important yeasts and moulds.

The three closing chapters will be found of especial value. One describes and discusses the methods and purposes of bacteriological examination of water, soil, and air, while the other two give an account of some valuable special methods which are used for particular purposes. The student will be glad to find here, in condensed but clear form, accounts of the methods of making tuberculin, diphtheria antitoxine, testing antitoxine, preparation and use of collodium sacs, of making the agglutination test, and other special topics of like character. These special methods greatly enhance the value of the work.

Dr. Novy has prepared this book designedly for students in medical schools, and has in general written in a clear, intelligible style. The work will be found useful to others besides medical students, and should find a place in all laboratories where bacteriology is studied. It will be a good book also to place in the hands of the student of general bacteriology. Although it combines some general discussion of bacteria with the directions for laboratory methods, its use must be practically confined to laboratory students.

H. W. CONN.

Wesleyan University.

**Whipple, George Chandler.** *The Microscopy of Drinking Water.* Pp. vii. + 300; woodcuts, 21; plates, xix. John Wiley & Sons, New York, 1899.

The experience which the author has gained as the former biologist of the Boston Water-works, and more recently as the biologist and director of the Mount Prospect Laboratory in Brooklyn, has given him unusual qualifications for the preparation of such a manual as this one. Heretofore, there has been no work in English devoted exclusively to the organisms found in water and to their influence upon the latter, as affecting its value as a source of supply for domestic purposes. It is likely that the book will be generally accepted as the authoritative discussion of the subject with which it deals. The work consists of two parts,<sup>ff</sup> the first dealing with the general biology of water supplies, the second consisting of a systematic description of the more important genera of aquatic microscopic organisms, exclusive of bacteria. The second part is illustrated by

nineteen plates containing about two hundred fifty figures. Four appendices deal respectively with the collection of samples; tables, formulæ, and reagents useful in the microscopic study of water; the bibliography of the subject; and a glossary of the technical terms used in Part II. The bibliography is very extensive and complete, containing nearly five hundred titles, which refer not only to papers in English, but also in German and French, and which are well arranged according to topics. There are a few important omissions, however. Surely, the list should include the published parts of the first volume of Engler and Prantl's "Die natürlichen Pflanzen-familien," containing as they do the Conjugate and Chlorophyceæ, with Schütt's treatise on the diatoms. And if volume one of the "Traité de Zoologie concrète" (La cellule et les Protozaires) by Delage and Hérouard be included in the list of references for "Protozoa," why should not volume five (Les vermiéens) of the same work be given in the list for "Rotifera"? The latter list is very meager, and the student will find this lavishly illustrated volume of the "Zoologie concrète" of much value. Again, the reference to Weltner's "Spongillidenstudien" (Archiv für Naturgeschichte, 1893, pp. 209-244) relates only to the literature of the Spongillidæ, while through some oversight, apparently, no reference is made to Weltner's own important researches which are given in the next part of the same "Archiv," pp. 245-284.

The subjects treated in the first part of the book are the history of the various earlier methods of examination; the object and methods of microscopical examination; microscopic organisms in water from different sources; limnology, geographical, seasonal, horizontal, and vertical distribution of microscopic organisms; odors of water-supplies; storage of surface water; storage of ground water; growth of organisms in water pipes. This portion of the book, as may be seen from the list of topics just given, will be of great value to water-works engineers, and sanitarians, containing as it does a brief and non-technical discussion of the trustworthy results obtained by the biologists and engineers of this country and of Europe. Much of the author's own original work is here given.

In the second part the system of classification of the larger groups followed is not such as is accepted by systematists in general, but rather one which the author regards as being more useful from the practical standpoint. The genera described are those more commonly found in the waters of lakes and rivers. Sanitarians will probably regret that the author has not stated in the description of each genus what is now known concerning its influence upon potable water. Owing to the method employed in reproducing the plates, many of the figures suffer from lack of clearness and detail. *Asterionella* (Pl. II, fig. 4) is represented as having the basal ends of the individual frustules united into a complete circle. This arrangement is, of course, impossible, since, owing to the diatom's mode of fission, the circle is always broken. A number of unimportant typographical errors occur, but these will undoubtedly be corrected in the future editions which the intrinsic value of the book will necessitate.

CHARLES WRIGHT DODGE.

**Phillips, O. P., Ph. M.** Immunity and the Rationale of Vaccination. Given before the Los Angeles County Med. Soc. and the S. Cal. Acad. Sc., Biol. Sect. Feb., 1899.

He reviews three methods of vaccination: by introducing living virus, by introducing a sterile toxine formed in an artificial medium, by introducing an

anti-toxine formed in some other animal. Attention is called to the danger of allowing the indiscriminate sale of anti-toxines, and the necessity of providing by law for their inspection, and for their return to the manufacturer when they have deteriorated through the action of heat, light, etc. The table gives the results of an examination, made in the laboratories of the University of Pennsylvania, of diphtheretic anti-toxines bought in the open market.

SERUM NUMBER.	UNITS CLAIMED.	UNITS FOUND.
3	500	345.
5	1000	647.5
6	1000	1380.
9	1000	177.5
11	1000	Less than 72.
15	1500	" " 410.
19	1000	1860.

E. M. BRACE.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

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### CURRENT LITERATURE.

**Nichols, J. L.** A Study of the Spinal Cord by Nissl's Method, in Typhoid Fever, and in Experimental Infection with the Typhoid Bacillus. Jour. Exper. Med., March, 1899.

The changes in the motor cells of the ventral horn and the nerve cells of the posterior root ganglia were studied in three cases of typhoid fever in man,

and in seven cases of experimental typhoid infection in rabbits. All the cases in man were severe infections. One died on the tenth, one on the eleventh day, and the third after an illness of two months. The typhoid bacillus was obtained in cultures from the organs in all cases. In two cases the autopsy was made six hours after death, and in the third fourteen hours. The changes were most marked in the ventral horn of the lumbar region. Both cells and dendrites were increased in size. The chromatic substance (Nissl's granules) were broken up and partially or completely dissolved. This process started near the nucleus and advanced toward the periphery of the cell. In some cases where this chromatolysis was most marked the nucleus had migrated to the opposite side of the cell. In these cells the slightly enlarged dendrites were almost free from chromatic substance. Changes in the achromatic substance were slight.

The changes were most marked in the case of two months duration (as the lung of this case showed pulmonary gangrene, it is reasonable to suppose that the changes in the cord were not entirely due to typhoid fever).



The changes in the dorsal root ganglia were slight, consisting in rarefaction of the chromatin, and changes in the shape and position of the nucleus.

In the rabbit, changes very similar to those seen in the cord of man were found.

In rabbits killed on the twenty-third and sixty-fourth days, respectively, it was thought that a process of restitution, as indicated by properly staining nuclei and a typical arrangement of newly formed Nissl's granules, could be made out.

In prolonged cases a degeneration of peripheral nerves (sciatic and its branches) was observed. The writer believes that these changes are quite common in typhoid fever, and that to them are due the post-typhoid hyperæsthesiss and paralyses.

R. M. P.

**Weiner, G.** Ueber ein Melanosarkom des Rectums und die melanotischen Geschwülste im Allgemeinen. *Zeigler's Beiträge*. 25: Heft II.

In this study a primary melano-sarcoma of the rectum in man is compared with two similar growths from the horse.

In the latter the growth does not penetrate deeply, and metastases are fewer in number. The growth is harder, firmer, and the nodules are not so liable to softening. There is no perivascular arrangement of the cells, all of which are pigmented. They are much less malignant. Tests for iron to ascertain if the pigment was hæmatogenous in origin, were negative.

In man the blood vessels are involved either by a perivascular growth, invasion of the vessel wall, or by actual penetration of the intima and projection into the lumen. Metastases take place generally through the blood stream, and are most common in the brain and the liver.

A. M.

**Malkoff.** Ueber die Bedeutung des traumatischen Verletzungen von Arterien (Quetschung, Dehnung) für die Entwicklung des wahren Aneurysmen und des Arteriosklerose. *Zeigler's Beiträge*. 25: 1899.

Malkoff has succeeded in producing aneurisms experimentally in the dog. He used two methods, compression by forceps and distension, as result of

injecting salt solution. The most marked changes were obtained in the compression experiments. Tears occurred in the intima, elastic plate and media, and a spindle-shaped aneurism developed. After forty days the lumen became normal, or smaller than normal. The lumen was markedly thickened and showed newly developed elastic fibers. The elastic fibers appear to develop from the periphery of the cells. There was an increase of connective tissue in the media. Deposition of lime salts were found as early as twenty days after injury.

J. H. P.

**Pio Foa.** Beiträge zum Studium des Knochenmarks. *Zeigler's Beiträge*. 25: Heft II.

In this study, which deals chiefly with giant cells, Flemming's and Hermann's solutions were used as hardening agents; and for staining, the methods recommended by Van Gieson, Mallory, and Ramon y Cajal. Mallory's stain gave the best results for the reticulum of the bone marrow, and for differentiating the protoplasm of the giant cells.

The giant cell is described as having three layers, an inner finely granular protoplasm surrounding the nucleus, an intermediate layer of thick fibrillar

protoplasm, and an outer layer made up of a reticulum of thread or cilia-like processes. Multiplication of the nucleus takes place by direct division, and is to be considered as a retrograde process. The giant cells take no part in the formation of blood cells. That the giant cells occasionally contain a polymorphonuclear leucocyte, indicates a phagocytic action on the part of the giant cell, and not an invasion of degenerating giant cells by leucocytes (Heidenhain's theory).

In experimentation on animals it was found that this phagocytic property of the giant cells was increased by suppuration, inanition, exhausting hemorrhages, and by intravenous injections of lecithin, milk and filtered cultures of the pneumococcus, and staphylococcus pyogenes aureus.

Foa believes that the giant cells are formed from the true marrow cells, which have much protoplasm, and a single large vesicular nucleus, with deep staining border in which is a prominent nucleolus. In these cells he has often observed various grades of nuclear division. The article is illustrated by two plates, showing thirteen figures.

R. M. P.

**J. K. M. Knox, Jr.** Supra-arterial Epicardial Fibroid Nodules. *Jour. Exper. Med.* 4: No. 2.

These fibroid nodules have been observed by Knox in five cases.

Although they are not uncommonly met with, they apparently have not hitherto been fully described. They occur in the epicardium over the coronary arteries and their branches, but are entirely outside the adventitia. They are described as small greyish-white semi-translucent or opaque tubercle-like bodies varying slightly in size. Histologically they are composed of dense fibrous sclerotic tissue, poor in cells. In the earlier stages of their development they contain fairly numerous lymphoid cells and fibroblasts. In the arterial wall beneath the nodule the muscular coat is often degenerated and thinned. The elastic coat is also much altered. This weakening of the wall would tend to allow a bulging of the artery if there were not some restraint. The formation of the dense fibroid nodule in the epicardium, the writer believes, offers this restraint.

R. M. P.

**Hoen, A. G.** On a Form of Degeneration of Striated Muscle Met with in the Uvula. *Jour. Exp. Med.* 3: 449-570, 2 pls., 1898.

Normal uvulæ were examined and compared with those which had relaxed and elongated. Two of these were from

patients who were habitual smokers. There was marked proliferation and polymorphism of the nuclei, as is usual in muscular degeneration. The nuclei are somewhat enlarged, but nearly normal in shape unless they become crowded. They may assume a great variety of forms. Some are spool-shaped, some are crescentic, and some with pointed ends are serrated, with the serrations corresponding to the transverse striæ of the fiber. Many have their long axes lying transversely to the fiber, and most are surrounded with a vesicle of homogeneous material which has a delicate limiting membrane, and cannot be stained. As the fiber degenerates pigment is deposited in and around the nucleus. During the pigmentary stage the nuclei tend to fragment, and become irregular in outline, triangular, knobbed, and honeycombed.

The muscle fibers thicken, measuring from .035 to .05 mm. as compared with

.019 to .023 mm. in the normal fiber; there are wavy, undulating lines, instead of the longitudinal striæ, and the individual fibrillæ become twisted upon each other like the strands of a rope. This occurs after the marginal deposit, which culminates in the vesicular formation surrounding the nuclei. In the final stage of degeneration there is a cylindrical plug of large vesicles containing the distorted nuclei and particles of pigment, through which a shadowy longitudinal line shows the position of the former fibril. Over one hundred slides were examined without finding any trace of regeneration.

The material was fixed in Zenker's fluid, five per cent. formalin, and mercuric bichloride. Celloidin was used for imbedding, and sections were stained in Delafield's hæmatoxylin, Weigert's fibrin stain, Van Gieson's picric acid-fuchsin, Ehrlich's triple stain, and a one per cent. aqueous solution of thionin. Glycerine-Heidenhain-hæmatoxylin was also used. Sections are stained in this from twelve to twenty-four hours, and do not need to be decolorized. E. M. BRACE.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace, Biological Laboratory,  
University of Rochester, Rochester, N. Y.

**Huber, G. Carl.** Observations on the Innervation of the Intracranial Vessels. Jour. Comp. Neur. 9: 1-25, 1 pl., 1899.

The results of the experiments oppose the views of physiologists as summarized by Hill, who says: "No evidence

has been found of the existence of cerebral vaso-motor nerves."

Experiments were made upon dogs, cats, and rabbits. There are both medullated fibers (sensory) and non-medullated fibers (vaso-motor) in the pia mater. The bundles of medullated fibers wind around the arterial vessels in long sweeps, dividing where these vessels divide, and exchanging fibers so that a wide-meshed network is formed. The fibers branch off at the nodes of Ranvier, and the division into smaller and smaller medullated fibers continues until the branches terminate in fine varicose non-medullated fibrils, which usually end in a small granule. Non-medullated fibrils also proceed from the medullated fibers at the nodes of Ranvier. Many of these terminal branches are thought to terminate in the adventitia of the vessels, or in the fibrous tissue surrounding them. No medullated fibers were found accompanying the veins of the pia.

There is also a perivascular plexus of non-medullated (vaso-motor) fibers which was found from the larger vessels, constituting the circle of Willis, to vessels with a muscular coat of not more than two layers of involuntary muscle cells. These resemble the vaso-motor nerves found in the wall of vessels in other parts of the body. Within this perivascular plexus there is a second plexus, not as well defined, which seems to lie just outside of the muscular coat. The termination of the ultimate fibrils of the plexuses may be seen in the muscular coat of the pial vessels. These fibrils run parallel to the long axis of the involun-

tary muscle cells, and in fortunate sections may be seen to give off short lateral twigs, terminating in fine granules in the muscle cells.

The same distinction is made as to the character of the medullated and non-medullated fibers of the dura mater. The medullated fibrils, after branching at the nodes of Ranvier, terminate in long varicose fibrils, extending through several fields of the microscope. These fibrils interlace, but do not anastomose. The sympathetic nerves form perivascular plexuses comparable with those found in other parts of the body.

*Technique.*—The animal was anesthetized, the canula inserted cerebralward in a carotid, and enough one per cent. methylen blue, made up in normal salt, injected to tinge the ear and eye of the side injected. After thirty or forty-five minutes, the brain and cervical cord were exposed with the least injury possible to the dura.

After removal, the brain was exposed to the air until the nerve fibers were stained, and pieces of the cortex were cut off with curved scissors and crushed under a cover-glass until the brain tissue was pressed away from the pia mater.

E. M. B.

**Obersteiner, Heinrich.** The Maintenance of the Equilibrium as a Function of the Central Nervous System. *Am. Nat.* **33**: 313-329, 1899.

Touch sensations, which are perceived by means of nerves from the joints, tendons, and muscles, sensations from the labyrinth of the ear, and optic sensa-

tions, react through the brain upon the muscular system to produce equilibrium. As a rule, we are unconscious of many of these sensations.

A parallelism is found between the size of the cerebellum and the delicacy of the muscular coördination. In mammals and birds, where very sensitive and complicated muscular action is required to maintain equilibrium in standing, running, or flying, the cerebellum is relatively large, and its surface is increased by many deep furrows. In amphibia and many reptiles which have merely crawling or jumping motions, the cerebellum is reduced to a mere ridge.

If the cerebellum of an animal is wounded badly, or if a portion of it is extirpated, there is no proper regulation of muscular contraction, and there is a decided disturbance of the equilibrium, although the intelligence of the animal is not interfered with. He believes that sensations are received in the cerebellum, and are there combined into a resultant nervous impulse which modifies the movements incited by the cerebrum in such a way that the desired coördination is attained.

E. M. B.

**Weil, Richard.** An Anomaly in the Internal Course of the Trochlear Nerve. *Jour. Comp. Neur.* **9**: 35-37, 1 pl., 1899.

The trochlear nerve in a human fœtus of between eight and nine months development was found to originate

normally in the floor of the Sylvian aqueduct, but instead of making its exit at the level of the decussation in the posterior medullary velum, it turns and runs forwards and outwards, traversing the velum and the posterior quadrigeminal bodies, and makes its exit between the posterior quadrigemina and the inferior fillet. Although this does not correspond with any stage in normal development, it is suggested that it may be taken as a clue to the original course of the nerve.



If we consider that it is a mesencephalic segmental nerve, the anomaly would be imperfectly atavistic. If it belongs to the post-mesencephalic segment, the nucleus of origin must have been displaced forwards. If the second primary cerebral incisure has no real segmental value, the nerve would be within the limits of a single segment, and the anomaly would have no significance.

E. M. B.

## NEWS AND NOTES.

A BRYOLOGICAL MEMORIAL MEETING AT COLUMBUS, OHIO.—Columbus was the home for many years of William S. Sullivant and Leo Lesquereux, two names which will always awaken love and reverence from all students of North American mosses and hepatics. It is twenty-six years since Sullivant died, and this last quarter of the century has seen a marked extension of the limits of bryological study and a large increase in the number of students. It seems a fitting time and place to take a survey of the field, review the past, and make plans for the future; hence it is proposed to make the coming meeting of the American Association for the Advancement of Science, which is to be held at Columbus, the occasion for a Memorial day in honor of the Nestors of American bryology, and to call on all botanists and scientific magazines to help make the occasion a memorable success. It is proposed to present a series of papers illustrated by specimens, photographs, microscopical slides, as well as by books and pamphlets, and to show the work of Hedwig, Palisot de Beauvois, Michaux, Muhlenberg, Bridel, Torrey, Drummond, Hooker and Wilson, Greville, Sullivant and Lesquereux, James and Watson, Austin, Ravenel and Garber, Rau and Wolle, Brandegee, Eaton and Faxon, Müller and Gottsche. Supplementing these, there will be shown collections of specimens macroscopic, and microscopic, illustrating the monographic work of living American students. Foreign students who have worked on North American mosses will be asked to coöperate with us. An effort will be made to secure the loan of type specimens and illustrations from the various public and private herbaria and libraries, both here and abroad, as well as from private collections. It is also intended to exhibit any portraits, autograph letters, type specimens and drawings of special interest which may be loaned for the occasion, as well as presentation copies of books and pamphlets. The following committee of organization will gladly answer questions and give assistance to those wishing to contribute: Mrs. N. L. Britton, New York Botanical Gardens; Prof. W. A. Kellerman, Ohio State University; Prof. Charles R. Barnes, University of Chicago; Dr. George G. Kennedy, Readville, Mass.; Prof. L. M. Underwood, Columbia University.

The coming meeting of the American Microscopical Society at Columbus, O., August 17 to 19, will certainly be one of the best in the history of the society. The practical character and number of the papers already presented to the committee are in keeping with the rapid growth of the use of the microscope in all branches of science. Directors of laboratories, their assistants, medical men, and investigators, will find in this meeting material which is not found elsewhere, and which is very important to them.

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## The Conditioning of Wool and Other Fibers in the Technological Laboratories of the Phila- delphia Commercial Museum.

### SECOND PAPER.

The epithelial scales of the wool fiber, mentioned in the preceding paper (Jour. App. Micr. 2: 289-292, 1899), are very irregular (Fig. 2), and the pressure of the cover-glass seems to have some influence, causing, here and there, elongations during the flattening; but it has been clearly established that the Merino breed, and the first ten crosses have a form of epithelial scale distinct from that of other breeds, and by studying these differences we may be able to learn their characteristics as distinguished from those of other breeds. The Merino scales are widest or broadest in the line of circumference of the fiber, but extremely narrow in the line of axis of the fiber. The scale of a coarse wool fiber offers a very interesting contrast. The separation of these epithelial scales is at all times a very delicate process, and is at first attended with little success, the observer being unable to make solar projection drawings which would give an approximate idea of their real character, but, with a little practice upon one and the same fiber, each operation will teach more and more of how and what to look for. We notice in Fig. 2 that some of the scales show what appears to be the nuclei to which much attention has been given, but as yet no positive conclusion has been reached, because there is still a possibility that these apparent nuclei are caused by extraneous matter, although great care has been taken that the fibers, before dissolving or separating the scales, should be freed of yolk, fat, natural suint, and foreign matters. Hence any suggestion as to the possibility of fatty globules being still present, must be dismissed in favor of the nucleus theory. The separating of the scales accomplished, we will now try to discover if possible the manner of attachment of these scales to the fiber proper. For that purpose we will take again a fiber of the Merino blood, viz., the Cotswold fiber, and with a B eyepiece, and a one-eighth objective, obtain an image of 800 X (Fig. 3). This is a typical fiber of the Merino-Cotswold breed. It shows the pointed and serrated edges of the trumpet-like epidermal scales which point upward, and are free for over one-half of their entire length, not unlike the growth of a rattan cane, especially near the root. Under the

microscope at the above magnification, the denticulated structure of the tooth-like scales is clearly seen against the opaque background, if an ordinary lamp with seven-eighths inch flat wick is made use of for the illumination. The light should, however, be indirect, and not pass through the image. Such a lamp is even better than the electric incandescent lamp, and is much more convenient.

This Cotswold-Merino fiber had been specially treated in 10 per cent. sodium hydroxide (NaOH) for one minute, washed in cold distilled water, carefully dried between blotting papers, and finally in air at 100 degrees F., and then mounted in 5 per cent. glycerine. It must be taken into consideration that the foregoing process, a kind of destructive treatment, was intentionally adopted



Fig. 2.—Epithelial Scale.

in order to bring out the scales more prominently by partially dissolving the albuminous structure of the fiber by the action of NaOH solution. We notice on the lower extremity of each scale, a very thin, membranous tissue, attached to the inner wall of the scale, which extends, also, directly to the cellular tube or fiber proper. We have also observed that these scales are sensitive to dry and moist atmosphere. They expand and contract, and this may be the reason why wool in fiber, or in cloth garments, absorbs from 6 to 19 per cent. of moisture, and no matter how dry such

wool may be, such absorption of water is persistently retained by the wool. But it must not be understood that these scales are fastened in the manner here suggested. It is, however, a theory resulting from many experiments and tests made with a view to finding a solution for these armor-plate-like coverings in structures which build up the so universally useful fiber of wool. The question arises, why are these scales serrated? To which we cannot find an answer. It is a fact that all hair, and wool, of man and animals, is more or less serrated, but the wool, and especially the finer classes, such as the Saxony and the Merinos, is the most prominently serrated of all. Next we notice the inner cellular cavities here and there filled with granules of pigment, and mineral salts, which give the various colors to hair and wool, and hence we call these cavities pigment cells. If we take an ordinary white wool, and boil a sample for the purpose of dissolving its tissues, we will get a whitish, albuminous kind of fluid from which no traces of pigment can be separated. But were we to treat a sample of mountain sheep wool, or of Scottish black-face, we would find a decided black pigment corresponding, in fact, to the various colors of the hair, or wool in its natural state. We will here quote a typical analysis of raw wool which shows that wool is by no means a simple substance. This analysis is one of many we have made on raw wool, and represents a very fair average :

Mineral matter, . . . . .	5.8
Suint and fatty matter, . . . . .	45.2
Pure fiber, . . . . .	36.6
Moisture, . . . . .	12.4
	<hr/>
	100.00

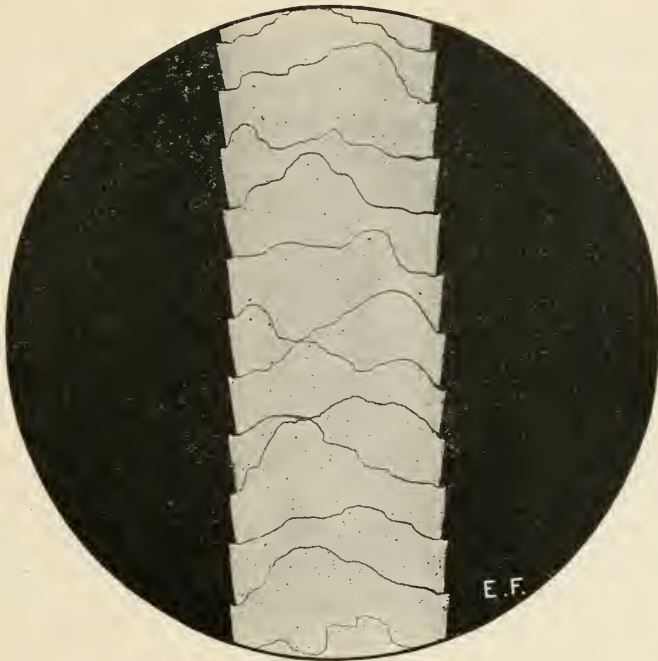


Fig. 3.—A Cotswold Fiber at 800 X.

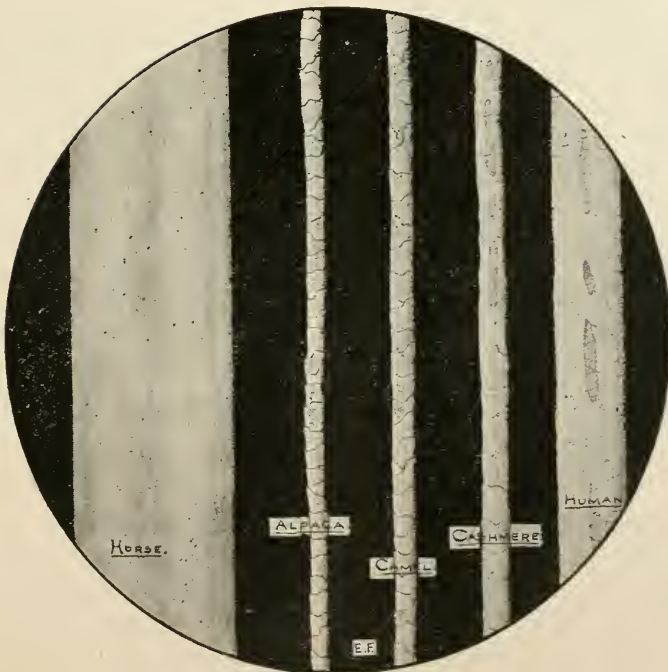


Fig. 4.—Characteristics of Different Fibers at 120 X.



The mineral matters are sulphur, calcium, magnesium, iron, manganese, silicates.

The yolk, or natural suint—wool fat—contains, besides the fatty, saponified matter, soda and potash, which the animal, during natural feeding, absorbs from the plant. We have said that wool, like hair, horns, hoofs, and feathers, belongs to the albuminoid family, and as in all living animals, the albumin, casein, and fibrin matters constantly change among themselves, from one into another, their specific analyses would differ but little. To illustrate our meaning more fully, we give an analysis of mixed hair, wool, and feathers, and horny substances—second of pure wool:

	No. 1. Mixed Hair, Wool, Feathers, Horny Substance.	No. 2. Pure Wool.
Carbon, . . . . .	50.45 . . . . .	50.20
Nitrogen, . . . . .	16.60 . . . . .	17.45
Hydrogen, . . . . .	6.24 . . . . .	6.60
Oxygen, . . . . .	23.21 . . . . .	21.85
Sulphur, . . . . .	3.50 . . . . .	3.90

We notice but a slight difference, and not sufficient to distinguish between the two. The ash of these samples represented 1.85 per cent. and 2.1 per cent., respectively, and is here accounted for along with the carbon. Test No. 2: Before analysis the wool was washed in lukewarm, distilled water, and dried at 110 degrees F. for three hours. Doctor Kleiner once recorded a sulphur analysis of 9 per cent. in Saxon-Merino wools. We have never been able to find more than from 2.50 per cent. to 4 per cent., whereas the yolk suint, and natural wool fat varies with the district, the climate, and the breed; it ranges between 12 per cent. and 65 per cent. of the weight of the wool; the amount of potash varies inversely according to the amount of fat or yolk, i. e., the higher the percentage of fat or yolk, the lower the percentage of potash. A low percentage of grease invariably means a higher percentage of potash. Nature seems to have provided these exudations as a protection from the weather. In the tropics the sheep breeder pays great attention to artificially supplying the yolk, or grease to the sheep in a preparation consisting of butter and oil, potash and tar, saponified into a kind of green soap, which is put on the backs of the sheep. The uses of the microscope in studies of this kind, however, are so many and various that their further consideration may be continued profitably at another time.

ERNEST FAHRIG,  
Chief of Laboratories.

WILLIAM P. WILSON, SC. D.,  
Director.

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To obtain expanded specimens of hydroids or other small forms, draw nearly all the water from the watch-glass containing them, and when they have expanded again, pour hot corrosive sublimate over them. If this is done quickly, the organisms will be fixed before they have time to contract. From two to five minutes is long enough to leave them in the sublimate, as the hot solution acts rapidly.

## The Preparation and Mounting of Wood Sections.

I commence this paper with the full realization that, by the side of highly specialized laboratory methods, I am writing in a very humble sphere, but I offer no apology. It is to be remembered that there is a great deal of valuable microscopical study that is not done in laboratories, and by those who have not the time or means for elaborate methods, nor is it to be forgotten that well prepared vegetable sections hold a large place in educational work.

The first thing required for successful section-cutting is a good microtome; no matter how cheap, or by whom made, so long as it does its work well. It is not every one that can train his nerves and muscles to cut free-hand sections of any size and uniformity of thickness. Good section-cutting means a knife of the finest steel, and the finest edge; the wake of "saw teeth" in an imperfectly sharpened knife never looks well in a wood section, and this sharpening of a knife is something that does not come of itself, it requires practice. No edge thin enough for fine sectioning will stand hard wood, unsoftened, and I have found nothing quite as good for this purpose as boiling for a short time, or soaking for two or three days in a strong solution of pearline; it softens without destroying any part of the tissue.

For imbedding use hard paraffin, especially in the summer; it shrinks less, holds the object more firmly, and the edges of material imbedded are less likely to tear. If the paraffin rolls, as it most likely will, press it lightly with the ball of the finger as the knife goes through it—no danger of sectioning the finger.

Next in importance is the right thickness of the section, which should be governed entirely by the object to be attained by observation, and not by the exercise of mechanical genius in cutting as thin as possible. Hard and compact tissue, like most of the woods, requires to be cut very thin, while objects like *Pteris aquilina*, root or stem, with large fibro-vascular bundles, require to be cut thicker to preserve the structure complete, and the section entire. This holds true especially of longitudinal sections. After cutting a few sections, examine with the microscope and vary the thickness, if necessary, till the desired result is reached; time spent in this way is time saved. It makes a difference whether the section is to be mounted in balsam, or some other medium, as will be seen hereafter.

If transparency is desired, most sections require to be bleached before staining. A good rule is to bleach till the chlorophyll disappears, or the color from the wood is discharged, and no longer. If bleached too little, the sections will be disfigured with dark blotches; if bleached too much, the section will go to pieces, or its character will be destroyed. The bleaching fluid must be well soaked out, in repeated changes of pure water, or staining will be a failure.

The primary object of staining, single or double, is not for beauty, but for use, and yet a beautiful slide is not to be despised on that account. Slides are *educators*, and the education of the sense of beauty is no barrier to scientific research, and it is quite possible that in some cases it may be an inspiration. It requires but little skill to so stain a wood section as to bring out stunning

colors, but that is not staining in any high sense of the word. Staining means such use of colors as to differentiate structure for accurate observation. And let me here say, that there are no line-drawn rules of universal application; it is largely an empirical art, and the best results, as a rule, are wrought out by careful and intelligent experimenting, and my aim is to offer a few simple and general rules which may serve as guides in lines of work within limited means and opportunities.

*Reagents.*—There is a long list of reagents in use, all of more or less value, but I mention a few only, with their use, which will be found most effective in the line of work before us; and I will preface this part of the subject by mentioning a single condition without which success is impossible, and that is *cleanness*. No dish, or glass, or instrument should ever be used without cleaning the last thing before using. Water, whether distilled or not, should be filtered, and all staining fluids will bear to be watched, and filtered as often as the least impurity appears.

For staining cell walls there is nothing superior to Delafield's hæmatoxylin, but nine-tenths of the staining done with this reagent is so deep as to render the object opaque and comparatively worthless. This fluid should be diluted with the purest water obtainable till the color is a very little deeper than that sought in the object, and allowed to stain slowly, or the section should have a very brief immersion in full-strength fluid—always rinsing in water. Hæmatoxylin has a delicate reaction, is permanent, and when properly used nothing is better for many things, if quite as good; but when unskillfully done, no staining is worse.

Bismark brown is a very valuable, but, as far as my observation goes, a very much neglected stain. It is somewhat opaque, and therefore not adapted to compact tissue, but where lace-like structure, or large spiral or scalariform vessels are to be shown, it has no equal. In combined compact, and loose structure, it has all the striking, and brilliant effect of double staining. Use the same methods as with hæmatoxylin.

The easiest and most effective double staining is done with red and green, and of these colors Grenacher's borax carmine, and methyl, or anilin green, will be found most generally reliable. Borax carmine is more properly an animal stain, but it is of the highest value in botanical work, when it plays its part in double stain. It requires time, and does not color deeply, but it is transparent, and can be made deep enough for all practical purposes if the following method is followed: first place the section in borax carmine for twelve hours or more, as found necessary; wash rapidly, yet thoroughly, in 50 per cent. alcohol; place for two or three seconds in a saturated solution of any of the anilin greens, preferably anilin or methyl; wash as before, and return to borax carmine till the red reappears, changing the borax carmine after the superfluous green is driven out. As soon as the outlines of the colors are distinctly marked, and secondary tints disappear, rinse as before, and place for a few moments in alum cochineal; this acts as a mordant to the borax carmine without destroying its transparency. This process, like any other, is liable to failure by over-doing or under-doing some part of it, but when successfully followed the result will be all that can be desired. It may seem superfluous to call attention to the chemical relations of

reagents, but in years of teaching I have found it necessary to guard this point with special care. Hæmatoxylin and alcohol must be kept separate or you will have more mud than color, and rinsing borax carmine with 95 per cent. alcohol will ruin everything by precipitation.

*Mounting Media.*—Which is the best, glycerine, glycerine jelly, or balsam? We will dispose of glycerine by counting it out entirely; it gives the poorest definition of all, and however well sealed, cells are liable to leak from expansion of the fluid by heat. The comparative merits of balsam and glycerine jelly are best determined by experiment. Balsam has three faults which render it objectionable where the fine details of structure are to be preserved. It shrinks protoplasm, and the process of dehydrating for mounting, distorts delicate tissue, and the medium makes it worse. Its tendency is to make everything transparent, and sometimes so transparent as to be worthless. There is an acid in balsam that is an enemy to color. The chemist that will furnish a neutral balsam will be a benefactor. Balsam has its merits, and is indispensable where the object is in itself opaque, or when outline definition mainly is wanted. It is easy to use, and the objection of transparency may be in part overcome by cutting the section thicker or staining deeper.

For mounting in balsam, dehydrate by passing quite rapidly through 50, 70, and 95 per cent. alcohol to absolute, and clear in oil of cloves, or in xylol which is better, if xylol balsam—which is to be preferred—is used.

Personally I prefer glycerine jelly to all other media for general use; it preserves structure and color best, and gives a character of honesty to the slide. Kaiser's formula is best as far as it goes, but to stand summer heat more gelatin should be used than the formula calls for, and it should be carefully and faithfully filtered. It is not as easily used as balsam, but little difficulty will be experienced if the following directions are followed:

Work in a warm room.

Heat, and keep hot while using, in a water bath.

Mount from chemically pure glycerine and 95 per cent. alcohol, one volume of each, kept till perfectly homogeneous, and filtered.

Warm the slide, and place the jelly on it with a glass rod, kept clean.

Place the object in the jelly, being sure that it is well covered. This may require an additional drop.

Hold the object in place and drain the slide to get rid of the glycerine and alcohol.

Cover the object again with jelly, and examine carefully under the dissecting microscope for air bubbles, especially for *stowaways*. Air bubbles should be worked off with a dissecting needle and not picked out with the forceps.

Cover the object again—take the cover glass between the thumb and finger—breath on it—cover it well with jelly—take it by the edge with the forceps—turn it over quickly—place it gently on the object at an angle, and apply a clip.

If it is desirable to mount more than one object on a slide, place them in just jelly enough to cover them safely from air, and give time to harden, after which an additional layer may be added, and the cover placed as before, and held with a clip. Next pass the slide over a spirit lamp, till the entire mass of jelly is



melted. If the right quantity has been used the objects will not slide out, or if they become displaced push them back again with a slip of pointed paper slightly moistened in the mouth. If the glycerine jelly becomes too thick by repeated heating, or from other cause, add a small quantity of filtered water.

Cottage City, Mass.

J. D. KING, PH. D.

## Method for Sectioning Eggs Containing Much Yolk.

Anyone who has worked upon eggs containing a great deal of yolk has probably encountered the difficulty of the yolk globules falling out of the section during the cutting, if the specimen has been imbedded in paraffin. To avoid this difficulty without having to resort to imbedding in celloidin, Hertwig used a method which has not, to my knowledge, been published. The stained specimen is imbedded in paraffin by the usual method, mounted upon the sectioning block, and upon the surface to be cut, a small drop of very thin collodion is placed; the greater part of the drop is then removed by gently touching it with the finger. Before cutting, it is necessary to wait a moment until the ether has evaporated, which may be hastened by blowing upon it once or twice. The section can then be cut with any ordinary paraffin knife, and thinner than if it had been imbedded in celloidin. The collodion must be applied in the manner described after each cut. Care must be taken not to have the layer of collodion too thick, or the section will wrinkle badly. A very thin layer suffices to hold the yolk globules in place. The sections are fixed to the slide, cleared and mounted by the ordinary paraffin methods. The advantages offered by the described method are: the section may be cut with any microtome knife or razor; it is not necessary to flood the knife with alcohol before cutting, and much thinner sections can be made by a less skilled workman.

CLARA LANGENBECK.

Wells College, Aurora, N. Y.

## A Convenient Method of Cleaning the Percentage Tubes of the Haematokrit.

In using the percentage tubes of the hæmatokrit, it is absolutely necessary that they be perfectly clean. Since they have so small a bore, it is evident that they cannot be cleaned by any ordinary process. In the laboratories of Purdue University, the method used is as follows: immediately after making a count, the tubes are placed in a dish of water for a few moments. This serves to loosen up what blood there may be in them. A horsehair loop, containing a thread, is then run through the lumen of the tubes. It has been found convenient to use this horsehair loop, as it is stiff enough, and of sufficient strength to carry the thread through. A needle might be used, but it is easier and takes less time to slip the thread through this loop than to thread the needle. After the tubes have been cleaned by the thread, it is necessary to dry them. In drying, a small bicycle pump, with a piece of rubber tubing fitted over the nipple to hold the tubes, is employed. In this way the tubes can be dried without any moisture getting into them. Both tubes can be cleaned and dried within five minutes, or less.

Purdue University.

CHARLES S. BOSENBURY.

## A Good Killing Fluid.

After experimenting with a large number of the various killing fluids which have been recommended from time to time, I have always come back to the chrom-acetic acid mixture as giving the most reliable results. Chrom-acetic acid is an excellent fluid for nuclear preservation, especially when the amount of acetic acid is relatively large. It is not so satisfactory for preserving the cytoplasmic structures. In finding a substitute for chromic acid, I have followed up the results of Tellyesniczky<sup>1</sup>, who recommends a mixture of acetic acid and potassium bichromate for preserving the structure of animal cells. After trying several strengths, I believe the following to be the best combination for plant cells: potassium bichromate, 0.8 gram; glacial acetic acid, 0.5 cubic centimeter; water, 99.0 cubic centimeters. This fluid gives excellent results. The objects do not become discolored, but remain clear. The nucleus is nearly, if not altogether, as well preserved as in chrom-acetic acid, while the cytoplasmic structures are usually more satisfactory. Small pieces of tissue, from a half to three-quarters of a centimeter long, should stay in the fluid from twelve to twenty-four hours. The sections take a good stain and are especially appropriate for anilin-safranin and gentian-violet, and Haidenhain's iron-alum-hæmatoxylin. Acetic-potassium-bichromate deserves a careful trial, as it will probably prove more satisfactory than chrom-acetic acid.

Ohio State University.

JOHN H. SCHAFFNER.

## A Modification of Van Gehuchten's Methelyn Blue Method.

The following is a modification of Van Gehuchten's methelyn-blue method as given by Prof. Lee. Excellent results have been obtained by it in the study of the brain of the fish, especially in the study of the cell structure.

Fish were killed by cutting the spinal cord just back of the medulla. The large blood vessels should be cut while the heart still beats, that as much blood as possible may be drained away from the brain. The blood corpuscles give a very deceptive appearance when stained in the vessels penetrating the brain. Before placing the brain in alcohol, it is well to cut away as much of the head as possible without injuring the brain. This allows the alcohol to penetrate uniformly and prevents the brain from becoming colored, as it does when the surrounding tissue is not removed. The head may then be placed in 35, 50, 70, 85, and 95 per cent. alcohols for from twelve to sixteen hours in each grade. The brain is now easily removed from the head and placed in absolute alcohol for four or five hours. It should *not* show any shriveled appearance, as it will if fixed in a high grade of alcohol at once. It is now put in chloroform until it sinks, when it may be put in the paraffin bath, and imbedded. Cut with a sliding microtome, the sections alternating 15 and 20  $\mu$ , since some points are more easily made out in the 15  $\mu$  sections, while others are best found in those 20  $\mu$  thick. Fix by the distilled water method. Remove the paraffin with

<sup>1</sup> Ueber die Fixirungs—(Härtungs)—Flüssigkeiten. Archiv. f. Mikr. Anat. 52: 202-247, 1898.

xylol and wash the slide with 85 per cent. alcohol for a minute or two to remove the xylol. Place the slide in the stain, which is prepared as follows: Dissolve 0.3 g. dry methelyn-blue in 250 cc. distilled water. Add to this 0.1 g. or 0.2 g. of any good uncolored castile soap. Allow this to dissolve and filter the mixture. Place the slide in a crystallizing dish which contains the stain. Set the dish on a bath or stove where its temperature can be kept at from 55 to 60 degrees C. Four or five hours is sufficient to stain the sections, although they do not overstain easily. A differentiating fluid is made from 90 parts absolute alcohol and 10 parts anilin oil. When no more blue color is given off, the sections may be cleared in oil of cajuput, and mounted in xylol balsam. So far, I do not find that this mounting medium affects the stain.

Nerve cells and their processes should show a rich blue stain, and their nuclei a yet darker stain. Fiber tissue should remain unstained, but can be followed with ease, as the tissues are well preserved. Fresh tissue is required to begin with, as old tissue *fails* of differentiation. The method applies equally well to the spinal cord, and to nerves. Good, clear outlines of ganglionic cells can be obtained, even with an oil immersion lens, in the 20  $\mu$  sections.

EARL RAMSEY.

Biological Laboratory, University of Indiana.

## A Convenient Source of Gregarinidae.

We may safely assume that nowadays every teacher of biology has had his students study amœbæ, for if he follow the directions of most guides, he cannot fail to find them. One such book naively directs: "Take a drop of water containing amœbæ . . .," in which case, of course, you cannot fail.

If it were more generally known how easily Gregarines may be obtained and kept "in stock," I believe they would be more often used for comparison, and as representatives of parasitic protozoa. Teacher-like, we gladly pass a good thing along.

Gregarinidæ may be found more or less abundant (usually less when you come to look for them) in the intestines of insects, and of other invertebrates. But in the alimentary tract of the yellow jointed larvæ of *Tenebrio molitor* they may be found at all times, and in abundance. This is the black beetle found in granaries, mills, barns, etc. Its larvæ, commonly known as meal-worms, and often erroneously called wire-worms, may be found in abundance in flouring mills, feed stores, under boards and bags that have lain for several months, or under feed-boxes in stables—anywhere, in fact, where ground grain is stored. The best time is when repairs are being made in a neighboring mill.

For keeping, place the larvæ in glass or stone jars, with plenty of the grain *débris* in which they are found, occasionally adding more meal, some dry-rotten wood, rags, etc., and they may be kept almost indefinitely without farther attention. If too well fed they metamorphose rapidly, at certain times of the year, and by keeping the jar covered, the beetles depositing eggs produce a new brood, but this is likely to deplete the stock.

At present I have a fruit jar half full of *débris* containing several hundred

larvæ that were collected over a year ago. In this time they have received a few dried thistle heads, some fragments of cork, bark, and rags. They are very hardy. Some were put with meal into a small tin can, tightly closed for transportation, but were misplaced, and not found until three months later. They were unharmed.

To find the Gregarinidæ for demonstration or study, snip off with small scissors both ends of a larva, seize the protruding (white) intestine with forceps, draw it out, and tease a portion in normal salt solution (water will do) on a slide. Cover, find with the low power (minute, oblong, transparent bodies), and study with any higher objective to suit. Now and then one larva, and sometimes several, may be found that have only a few or none of the parasites, but I do not remember ever to have examined more than three in succession without finding hundreds of them.

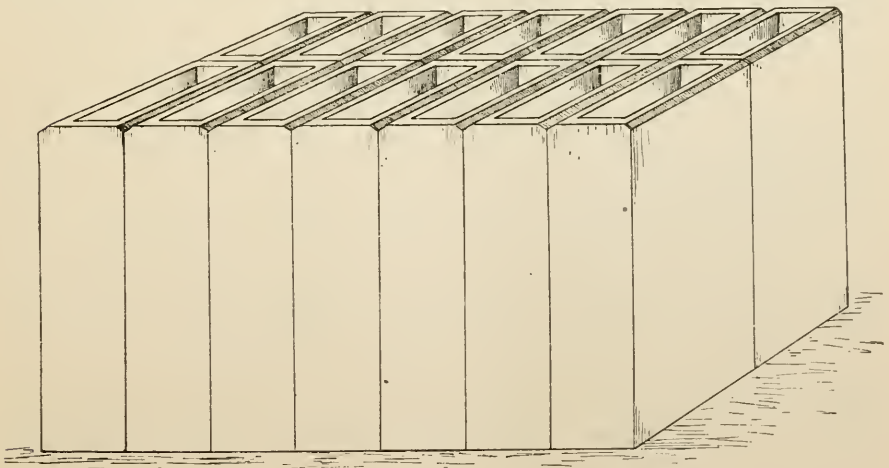
The larvæ, by the way, serve another purpose; they are used for feeding certain birds, chameleons, etc., so that in large cities they may be found for sale at bird stores.

L. MURBACH.

Detroit, Mich., May, 1899.


## A New Staining Dish.

A set of Stender dishes or Naples jars for staining sections on the slide occupies an undue amount of space upon the laboratory table, and requires a much larger quantity of the reagents than is demanded by the mere necessities of staining. An ordinary dish of the Stender pattern, large enough to take four or five slides without putting them back to back, requires about 120 cc. of the reagent; a Naples jar for two slides, placed back to back, requires about 40 cc. It is well known to histologists that in case of many reagents much more is spoiled or wasted than is used, e. g., 40 cc. of gentian-violet in a small Naples jar will last as long, and do as effective work as 120 cc. in a Stender dish, since in either case the stain will probably lose its efficiency on account of spoiling. The same is true of many other stains. There would be an equal saving in





alcohols, since for accurate work these usually have to be renewed on account of the coloration due to the stains, and not on account of any lack of effectiveness in the alcohol itself.

The figure represents fourteen staining dishes of a new pattern. The whole group occupies a space of only six and one-eighth inches by three inches, and each dish requires only 20 cc. of the reagent. Four slides may be stained at a time by placing them back to back. The tops of the dishes are ground smooth, and are covered by slips of ground glass (not shown in the figure), one and three-eighths of an inch long, and three-quarters of an inch wide. Although rather tall and slender, the dishes will stand alone, but it is better to surround the whole group by a strong rubber band. The interior of the dish is one and one-eighth inches long, one-half inch wide, and three and one-quarter inches deep. The bottom of the interior is not flat, but broadly -shaped. The economy in space is an essential point, and the saving in reagents would soon pay for the dishes.

CHAS. J. CHAMBERLAIN.

University of Chicago.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

### VI.

#### THE CELLOIDIN METHOD.

"Celloidin is a form of nitro-cellulose." It is very inflammable, but does not explode. It may be obtained in the form of tablets or cuttings, which have to be dissolved in a mixture of equal parts of absolute alcohol and ether. It is customary to use two solutions, a "thick" and a "thin." The thick solution (about 10 or 12 per cent.) should have about the consistency of thick syrup. The thin may be made by mixing equal parts of the thick and ether alcohol.

As mentioned in the chapter on the General Method, the killing, washing, and dehydrating are the same as for the paraffin method. After dehydrating in absolute alcohol the succeeding steps are as follows:

- (1) Ether alcohol, 1 to 2 days.
- (2) Thin celloidin, 2 to 6 days.
- (3) Thick celloidin, 3 to 10 days.

It seems better, however, to begin with about 2 per cent. celloidin and transfer successively through 4 per cent., 6 per cent., etc., to 12 per cent., or to allow the 2 per cent. to concentrate by removing the cork for a short time each day.

4. *Imbedding*.—The material may be imbedded and mounted upon a block at the same time. The blocks should have surface enough to accommodate the objects, and should be about one-fourth of an inch thick. Place the block for a moment in ether alcohol and then in 2 per cent. celloidin; with the forceps remove a piece of the material from the thick celloidin and place it upon the block, taking care to keep it right side up. Dip the block with its object first in thick celloidin, then in thin, and after exposing to the air for a few minutes drop it into chloroform, where it should remain for about ten to twenty hours. It

should then be placed in equal parts of glycerine and 95 per cent. alcohol, where it may be kept indefinitely.

In cutting, the knife should be set as obliquely as possible, and both the knife and the object should be kept wet with the mixture of glycerine and alcohol. The sections are transferred to 70 per cent. alcohol as fast as they are cut. The succeeding steps are the same as for free-hand sections, but many stains are not available because they stain the celloidin. Safranin and Delafield's hæmatoxylin, or Delafield's hæmatoxylin and eosin are good combinations for celloidin sections. Do not use absolute alcohol for dehydrating, since it dissolves the celloidin, but transfer from 95 per cent. alcohol to Eycleshymer's clearing fluid (equal parts of bergamot oil, cedar oil, and carbolic acid), which clears readily from 95 per cent. alcohol. Mount in balsam.

The celloidin method has its disadvantages as well as its advantages. It is extremely slow and tedious, and it is rarely possible to cut sections thinner than  $10\mu$ , while, on the other hand, it gives smoother sections. The entire absence of heat makes it very useful for delicate, succulent tissues. Stems and roots which cannot be handled at all in paraffin, cut well in celloidin, and much larger sections can be cut than in paraffin.

When material is to be imbedded, use celloidin as a last resort. Use paraffin when you *can*, celloidin when you *must*.

I am indebted to my friend Mr. W. B. MacCallum for several suggestions in regard to this method.

#### THE GLYCERINE METHOD.

It is hard to get the filamentous algæ and fungi into balsam without shrinking; consequently, these forms are usually mounted in glycerine or glycerine jelly.

Flemming's fluid and chromo-acetic acid are good fixing agents. Corrosive sublimate in water, or in 70 per cent. alcohol, used hot, is also to be recommended. For general morphology, stain for six hours or over night in a one-half per cent. aqueous solution of eosin, transfer directly to a 1 per cent. solution of acetic acid in distilled water, and allow it to act for about five minutes, wash thoroughly in water to remove the acid, and then put the material into a watch glass in a 10 per cent. solution of glycerine in water. The watch glass should be kept as free from dust as possible, and should not be covered. As soon as the solution appears to be about as thick as pure glycerine the material is ready for mounting. Place a small quantity of the material on a slide, arrange it carefully, add a small drop of glycerine, and a round cover. Seal with gold size (a varnish used by painters in laying gold leaf). None of the sealing media will stick to moist surfaces, hence it is essential that there should be only enough glycerine to come to the edge of the cover. If it is desired to mount rather large specimens, like the antheridia, and the oögonia of *Chara*, it is best to spin a ring on the slide, thus forming a shallow cell.

If glycerine jelly is to be used, place the bottle in warm water until the jelly becomes liquid, but avoid any unnecessary heat. Take the material from the glycerine, add a drop of the warm jelly, and seal as before.

## MOUNTING DIATOMS.

The silicious shells of diatoms are among the most beautiful objects which could be examined with the microscope. To obtain perfectly clean mounts requires considerable time and patience, but when the material is once cleaned, preparations may be made at any time with very little trouble. Diatom enthusiasts have devised numerous methods for cleaning diatoms, and separating the various forms from each other, but we shall give here only a few simple, practical methods.

Many scouring soaps and silver polishes contain large quantities of diatoms, and the diatomaceous earths are particularly rich. Break up a small lump of such material and boil it in hydrochloric acid. A test-tube is very convenient for this process. Let the diatoms settle, pour off the acid, and then wash in water. As soon as the diatoms settle, the water should be poured off. The washing must be continued until no trace of acid remains. After the washing is complete, pour on absolute alcohol, and after ten or fifteen minutes pour off the alcohol, and add just enough xylol or bergamot oil to cover the sediment. The material, of course, will keep indefinitely in this condition, and may be mounted in balsam at any time.

The method just given refers to fossil diatoms. A convenient method for mounting the frustules of living forms is given in the March number of this journal.

If it is desired to bring out the nucleus, centrosomes, and chromosomes, Flemming's fluid, followed by the safranin-gentian violet-orange combination, may be recommended. Haidenhain's iron-alum-hæmatoxylin is very good for the centrosome.

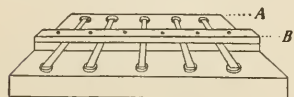
## MOUNTING BACTERIA.

The methods of modern bacteriological technique are so numerous and so specialized that we must refer to laboratory manuals for instruction in this subject. The method given here will merely enable the student to recognize the larger bacteria.

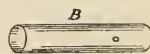
Foul water at the outlets of sewers and such places will usually afford an abundance of coccus, bacillus, spirillum, and bebbiotoa forms. Place a drop of the water on a slide, heat it gently until the water evaporates, then stain with fuchsin or methyl violet, clear in xylol, and mount in balsam.

*(To be Continued.)*

APPARATUS FOR HOLDING COVER-GLASSES AND CELLS IN POSITION.—A useful and simple piece of apparatus, that the students make for themselves, is used for fixing cells, cover-glasses, etc. It consists of an oblong board (A)



of well-seasoned mahogany measuring 12" x 9" x  $\frac{3}{4}$ ", down the center of which a strip of wood (B) one inch wide by three-eighths of an inch thick has been glued. Across the slip (B) is arranged, at right angles, a series of straight pieces of watch spring about four inches in length, to the extremities of each of which a small circular pad of felt one-eighth of an inch thick is affixed. These springs are kept in place by laying a second strip of wood along the top of them and fastening them to B by means of screws.



J. H. COOKE.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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DURING the summer scientists have been carrying on investigations at various places throughout the country, and much of interest has developed in the course of their work. Men coming together from different localities have had opportunities to compare ideas, to test new methods, and to modify old ones, and aside from the theoretical bearings of their work there are many valuable suggestions as to how results are best obtained. The JOURNAL invites all to contribute notes on whatever they may have found to help them.

The success of the investigator depends primarily upon his methods; the advance of modern science has been largely dependent upon improved technique and better apparatus, and the final solution of many problems seems now to depend upon still greater perfection in these lines. If anyone has found a new method or some modification of an old method that is of value in his own work, it will be of value to some other student, and there is opportunity for much mutual aid by a comparison of difficulties and the way out of them. The JOURNAL invites correspondence from those who can give suggestions and from those who have questions to ask.

\* \* \*

THE opening this year of several new biological laboratories for investigators shows an increased interest in research. The teacher of science must face the double problem of teaching his subject, and of finding new facts related to it. While desiring to find the best method of presenting the subject to students, he must consider the danger of neglecting the pursuit of new truths. Among many of our colleges even, there is a feeling that the professor ought to limit his work to the teaching of classes, and if he attempts anything further than this that he is perhaps defrauding the institution of a portion of his time.

If scientists in past times had been satisfied with presenting their subject in a forcible and attractive manner, scholars might still be using the Doctrine of the Spirits, with its theory of brain fluid circulating through tubular nerves and their continuous end-loops, to explain the phenomena of irritability, along with many other false principles. An occasional investigator may be able to devote himself to research, but the weight of responsibility for progress falls upon the teachers. The best of our universities recognize the importance of both phases of work and allow time for it, but more attention should be paid to it by all institutions of learning. In their application to medicine and other professions, the Roentgen rays have been of the greatest value, but they were discovered and their use made possible by purely experimental work. The discovery of bacteria revolutionized the science of medicine and surgery, was the starting point for new industries, and gave us a better understanding of the organic world. There is a vital relation between investigation and teaching that makes it of the highest importance for the scientist to do his share in both fields.



## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

Dixon, H. H. The Possible Function of the Nucleolus in Heredity. *Ann. Bot.* 13: 269-278, 1899.

The suggestion contained in the title is merely a tentative hypothesis which accepts the theory that hereditary sub-

stance is completely contained in the chromosomes during nuclear division, but adds that, during the resting stage of the nucleus, the hereditary substance may be distributed between the chromatin thread and the nucleoli. Professor Dixon produces considerable evidence in favor of this view. The opinion that in early stages of mitosis the substance of the nucleoli is distributed along the chromosomes is gaining ground, as repeated observation shows that when the amount of chromatin increases, the amount of nucleolar matter decreases, and *vice versa*. In regard to the equivalence of the male and the female hereditary masses, it is known that just before fertilization, the male nucleus is often a compact mass of chromatin, with no trace of nucleolus, while the female nucleus has very little chromatin, but has a large nucleolus. It frequently happens that this difference is equalized before the union of the germ nuclei, the quantity of chromatin increasing in the female nucleus evidently at the expense of the nucleolus, while in the male nucleus, nucleoli appear at the expense of the chromatin. The bearing of such a theory upon the reduction of the hereditary mass is then discussed, and it is suggested that the extrusion of nucleolar substances during the development of sexual cells may be concerned in this process. The writer believes that his theory is supported by Shaw's work on *Onoclea*, by the work of Farmer, Williams, and Strasburger, on *Fucus*, by Wager's work on *Saccharomyces*, and by other evidences. The theory is certainly suggestive, and will doubtless lead to a more definite knowledge of the nucleolus, whether the theory itself proves to be true or false. C. J. C.

Lounsberry, Alice. A Guide to the Wild Flowers, with an introduction by Dr. N. L. Britton. 8vo. pp. XVII+347. Sixty-four colored and one hundred black and white plates, and fifty-four diagrams, by Mrs. Ellis Rowan. Frederick A. Stokes Company, New York, 1899.

The attention which is being paid to nature study in the public schools has created an imperative demand for literature on this subject, but many who have attempted to supply the demand have had such a

meager knowledge of facts that their books must be regarded as fairy tales of no scientific value, and of no use as a guide to study in the field. The present book is admirably planned, the arrangement being determined by ecological relations. The principal topics are: Plants growing in mud, bogs, swamps, and marshes. Plants growing in light soil, sandy soil, dry soil, open woods, upland places, thickets, meadows, waste soil, roadside banks, and lanes. In discussing any particular flower, the writer gives both the common and

the scientific name, the family, color, odor, range, and time of bloom, a brief description to aid in identifying, and finally some account of the plant's habits, habitat, and folk-lore or literary allusions. It is to be regretted that there are frequent lapses into a gushing sentimental style, which detract much from the beauty of the book. There are indices to color, English names, Latin names, and technical terms. In speaking of the cardinal flower, the writer says, that "The generic name, lobelia, has become so familiar that we use it freely, and are unconscious of its being more difficult to manage than the common name. In this connection it comes to the mind to ask if all the botanical names would not become equally simple if we would put ourselves on closer terms of intimacy with them." This point is certainly worth the attention of teachers. The large number of illustrations gives the book an attractive appearance, and adds greatly to its value. The work is instructive and suggestive, and is worthy of the attention of those who have to teach nature study. C. J. C.

**Jeffrey, E. C.** The Development, Structure, and Affinities of the Genus *Equisetum*. Mem. Bost. Soc. of Nat. Hist. 5: 155-190, pl. 26-30, 1899.

This paper is devoted to the development and structure of *Equisetum*, but also discusses related forms, both living and fossil. It is suggested that

too much attention has been paid to the first division of the ovum, while the later stages, when differentiation of organs and tissues are in progress, have been comparatively neglected. The following are the important conclusions: In the development of the vascular axis of the young plant, there are two primitive types of axis, one with a single concentric bundle, and the other with the vascular tissue from the outset in the form of a tube. In regard to the general morphology of the vascular strands, Mr. Jeffrey returns to the standpoint of Sachs and DeBary. The prothallia of *Equisetum* bear a striking resemblance to the prothallia of certain species of *Lycopodium*, while the archegonium, being without a basal cell, resembles that of certain isosporous *Lycopods*. The embryos, as far as studied, agree with those of *Lycopodia* as described by Treub. The reduced leaves and the strobiloid fructification also agree with *Lycopods*. A study of fossil allies leads to the conclusion that *Lycopodiales* and *Equisetales* are closely allied, and that the *Sphenophyllales* must be included with the *Equisetales* as an additional order. Twenty-four of the illustrations are from photomicrographs. C. J. C.

**Snow, Julia W.** *Pseudo-Pleurococcus*, Nov-gen. Ann. Bot. 13: 189-195, pl. 11, 1899.

The question of polymorphism in the genus *Pleurococcus* has some new and

valuable light thrown upon it by Dr. Julia W. Snow, in an article appearing in the *Annals of Botany* for June. This writer uses pure cultures, after the manner of Klebs, and the nutrient solution of Knop in divers concentrations, with and without a thickening of agar. The external characteristics of many *Algæ* may be changed by varying the nutrient medium, but although she has kept pure cultures of a number of forms growing for many months at a time, the writer has never been able in this way to produce any change in the internal characteristics. These, according to all her observations, remain quite constant, no matter how the external form of the plant may be altered by

changed environment. Hence she believes that these internal characteristics of the cells form a surer ground for judging of relationships than any other. Klebs, Artari, and Gay maintain that *Pleurococcus vulgaris* is a constant form, either unicellular, or forming small, more or less quadrangular cell-complexes, without pyrenoid, and with a chromatophore lining the whole cell-membrane. But Chodat includes in this species forms with and without pyrenoid, and with either parietal or stellate chromatophore. On account of the general observation stated above, the work is in accord with that of Klebs, Artari, and Gay. But forms are found which, in the unicellular condition are hard to distinguish from *Pleurococcus vulgaris*, and which do produce filaments when placed in a liquid medium. However, these forms cannot be called *Pleurococcus vulgaris*, since in them the chromatophore lines only a part of the cell-membrane, and they possess pyrenoids. There is also a remarkable physiological difference between the true species and these filamentous forms; the former soon dies in Knop's solution, while the latter live indefinitely therein. Thus these filamentous forms are to be classified, not as *Pleurococcus vulgaris*, nor—considering the pyrenoids—as *Pleurococcus* at all, but are to be put into another genus, and since these filaments have not produced zoöspores, the writer believes it to be better to create a new genus for them than to follow Klebs, and put them into the genus *Stigeoclonium*. For this new genus she suggests the name *Pseudo-Pleurococcus*, and gives descriptions of two species under it.

The genus *Pseudo-Pleurococcus* Snow is made up of forms which, in the atmosphere, are either unicellular, or form parenchymatous masses; in liquid media they form filaments. The chromatophore lines a part of the cell-membrane, but not all. *Pseudo-Pleurococcus botryoides* Snow was found on tree trunks near Ann Arbor, Michigan, while *Pseudo-Pleurococcus vulgaris* Snow was gathered from a similar habitat in Basel, Switzerland. The main differences between the two species are these: in the former the branches arise laterally on the filaments, in the latter they originate by a forking of the terminal cell; in the atmosphere, the former produces larger cell-complexes; and in nutrient solution the former branches more profusely. The article is accompanied by a colored plate, showing various forms of both new species.

University of Chicago.

B. E. LIVINGSTON.

#### CURRENT LITERATURE.

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## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
 Agnes M. Claypole, Sage College,  
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## CURRENT LITERATURE.

**Gorham, F. P.** Some Physiological Effects of Reduced Pressure on Fish. Jour. Bost. Med. Soc. 3: 250-256, 1 pl., 1899. This paper embodies the results of some experiments made upon salt water fish kept in aquaria, to find the cause of a gas-forming disease which produces bubbles of gas in different parts of the body, sometimes filling the connective tissue of the orbits, and forcing the eyes from their sockets. The experiments also throw some light on the function of the air-bladder. Contrary to expectation, the disease was found not to be caused by bacteria, but to be due to change of pressure.

Normal fish, in an aquarium from which the pressure of air had been removed, showed all the evidences of the disease in from a few minutes to a few hours, while control specimens would remain free from the disease for several days. Fish already affected were cured by being placed under a pressure of twenty feet of water. Evidently, when a fish is under less than the customary pressure, the gas dissolved in the blood becomes free, gas in the air-bladder and tissues expands and seeks an outlet, and in this way the bubbles under the skin are formed. This explanation agrees with observations made on the effects of bringing deep-sea fish to the surface. The theory that the air-bladder enables the fish to rise or fall in the water by changing its specific gravity is no longer tenable. The gas expands, or condenses, as the fish comes to the surface, or sinks to a lower level, tending to carry it still farther up or down. Apparently the function of the air-bladder is to keep the fish at the same general level, although it can accommodate itself to slight changes by secretion, or absorption of gas by the walls of the bladder. The gases of the air-bladder are oxygen, nitrogen, and carbon dioxide. If gas is liberated within the cells, as appears probable, the process would be comparable with the liberation of oxygen from green plants, and the fixation of nitrogen by the symbiotic bacteria of the Leguminosæ. The subject offers interesting questions for investigation.

E. M. BRACE.

**Friedmann, Franz.** Ueber die Pigmentbildung in den Schmetterlingsflügeln. Archiv f. Mikr. Anat. 54: 88-95, 1 pl., 1899.

Observations were made upon the formation of pigment in *Vanessa urticae*, beginning four days after it had passed into the pupa stage. Specimens were fixed in Hermann's fluid—15 pts. one per cent. platinum chloride, 4 pts. two per cent. osmic acid, 1 pt. acetic acid—in which they were left forty-eight hours. The osmic acid produced a blackening of fat bodies, which are packed into the leucocytes found between the lamellæ of the wings, in the scale mother-cells, and along the edge of the epithelium.

These bodies are the early form of the pigment. During the development



of the butterfly, they pass from the leucocytes into the epithelium, and the scale mother-cells, either by becoming fluid, or by the amœboid powers of the cell. There is no penetration of the leucocyte into the scale, as stated by Mayer. After this migration the fat globule changes chemically, so that there is less reduction by osmic acid, and a brown instead of a black reaction is given. By the end of the fifth day the wings are laid in folds, and the color pattern is defined. The eleventh day after pupation, the globules pass into the scales, where they break up into powdery granules, forming the definitive diffuse pigmentation of the scales.

E. M. BRACE.

**Wilson, E. B.** The Structure of Protoplasm. This is one of a series of lectures delivered at the Marine Biological Laboratory at Wood's Holl. It is a study of the relation between the structure of protoplasm and its vital activities, more especially as shown in the echinoderm egg. Modern theories of protoplasmic structure are divided into two classes, the first agreeing with Klein and Van Beneden, that protoplasm consists of a reticulum imbedded in a homogeneous matrix; the second, the view of Bütschli, that it has an alveolar or emulsion-structure. Wilson, after studying the living eggs of *Asterias*, *Echinarachnius*, *Arbacia*, and *Ophiura*, revises his earlier opinions and agrees with Bütschli, that protoplasm in the resting cell is a mixture of liquids in the form of a fine emulsion. Seen in sections, it appears to consist of a meshwork of granules imbedded in a less deeply staining, continuous substance, and within the meshes is a third homogeneous, slightly staining, ground-substance. Studies on the living egg show that the appearance of the meshwork results from the alveolar structure, the granules being due to coagulation effects, and that all the elements of protoplasm, including the granules, are liquid. The larger drops of the emulsion determine the alveolar structure, and form the ground-substance in the spaces of the meshwork; the smaller drops form the microsomes. Both of these lie in a homogeneous substance which may really be an emulsion beyond the range of vision, for the limit of the size of the drops is arbitrary, depending upon the power of the microscope. There is reason to believe that the three parts of protoplasm are merely different gradations of one structure, although the parts differ chemically, and in other respects, in the same way as the larger masses to which they give rise.

The coarser alveolar structure may be considered as secondary in origin, for the young ovarian egg is a homogeneous mass in which the alveoli form later by growth and differentiation.

The astral rays are thought to be true branching fibrillæ built up at the expense of the continuous substance of the cell, and there does not appear to be sufficient ground for believing that they arise from a specific archoplasm.

It is difficult to make a sharp distinction between living and lifeless masses in the cells, and the terms active and passive are substituted. The homogeneous protoplasm may be composed of ultra-microscopic bodies, which produce the visible elements by growth and differentiation. If we consider them as protoplasmic units, life must be considered, not as a manifestation of them, but of the systems which they form.

E. M. BRACE.

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- Corning, H. K.** Ueber einige Entwicklungsvorgänge am Kopfe der Anuren. *Morph. Jahrbuch*, **27**: Heft 2, 2 pls., 1899.
- Minot, C. S.** On the Veins of the Wolffian Bodies in the Pig. *Proc. Bost. Soc. Nat. Hist.*, **18**: 1 pl., 1899.
- Weismann, A.** Thatsachen und Auslegungen in Bezug auf Regeneration. pp. 31, 1899.
- Jackson, R. T.** Localized Stages in Development in Plants and Animals. Pp. 65, 10 pls. Boston, 1899.
- Davenport, C. B.** The Role of Water in Growth. *Proc. Bost. Soc. Nat. Hist.*, **18**: 1899.
- Marengi, G.** Anatomia del corpo umano. Nozioné elementari, con prefazione di C. Golgi. Pp. 38, 19 pls. Milan, 1899.
- Hardy, W. B.** On the Structure of Cell-protoplasma. *Jour. Physiol.*, **24**: No. 2, 1 pl. May, 1899.

## CURRENT BACTERIOLOGICAL LITERATURE.

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**Pearce, R. M.** The Bacteriology of the Accessory Sinuses of the Nose in Diphtheria and Scarlet Fever. *Jour. Bost. Soc. Med. Sci.*, March, 1899.

In this report fifty cases clinically diagnosed as diphtheria or scarlet fever, and bacteriologically confirmed in the case of diphtheria by examination of the nose and throat, were examined in order to ascertain whether the antra and other accessory sinuses were involved. In none of the cases were there any symptoms pointing to disease of the antra or other sinuses. Three of the cases were adults aged respectively nineteen, twenty-two, and twenty-four years, two were ten years old, one twelve, and all the rest between two and six years. Most of the cases in which there was an invasion of the sinuses died in from four to ten days, the average being the ninth day. The earliest infections occurred in diphtheria on the second and third days after membrane formation. All the scarlet fever cases with disease of the sinuses died between the ninth and fifteenth days.

The cases are divided as follows: diphtheria, thirty-nine cases; diphtheria complicated by scarlet fever, five cases; diphtheria complicated by measles, two cases; scarlet fever, four cases. In the examination of the sinuses precautions were taken not to introduce organisms from the outside. Cultures were made from the contents of the sinuses, and controlled by smears, and infection of the frontal, sphenoidal, and ethmoidal sinuses was only accepted as conclusive when a definite exudate was found, and in which the results obtained by smears and culture were the same.

In twenty-five of the thirty-nine uncomplicated diphtheria cases, inflammatory changes were found in the accessory sinuses, as follows: both antra, sixteen; both antra, sphenoidal, and ethmoidal sinuses, two; one antrum only, five; sphenoidal sinuses only, five. Of the eighteen double antral cases the exudate, on both sides, in three was a thick yellow pus; in three, a sero-purulent fluid; in three, a thin, cloudy, serous fluid; in one, a purulent fluid with membrane; in one, a cloudy serous fluid with membrane; and in seven, a thin mucoid fluid.

Excluding the seven cases with mucoid exudate, the bacillus of diphtheria was present on both sides in all but three cases, and in two of the latter it was present on one side, the pneumococcus being found on the other. In the third only the streptococcus was found. In two cases, the diphtheria bacillus was the only pathogenic organism present; pyogenic cocci or the pneumococcus were associated with it in all the other cases. The streptococcus occurred in nine cases, the staphylococcus pyogenes aureus in three, the albus in two, the pneumococcus in three, and the colon bacillus in four. Of the seven cases of mucoid accumulation, two were sterile; one contained the streptococcus; one, the staphylococcus pyogenes aureus; one, the diphtheria bacillus alone; and two, the latter organism associated with the streptococcus and pneumococcus respectively. An infection of the sphenoidal sinus was also present in two cases of double antral empyæma. In one, with a cloudy serous fluid, the streptococcus and the bacillus of diphtheria were found; in the other, with a thick yellow pus, the streptococcus alone. Of five cases with infection of the antrum on one side only, two contained the bacillus of diphtheria and the staphylococcus pyogenes aureus; one, the latter organism with the streptococcus and the colon bacillus; one, the diphtheria bacillus alone; and one a variety of non-pathogenic organisms. In two cases, in which only the sphenoidal cavity was involved, the streptococcus was present in the one case, causing a general infection; in the other, infection of the middle ear.

In the two cases of diphtheria with measles both antra were involved, the diphtheria bacillus and the streptococcus being found in both. In one of these cases both middle ears were purulent, and contained the same micro-organisms as the antra. In three of the five cases of diphtheria with scarlet fever the antra were normal; one contained the diphtheria bacillus with some unrecognized bacteria in one antrum. In the second case one antrum furnished cultures of the streptococcus, the other of the streptococcus and the staphylococcus pyogenes aureus. In this case the sphenoidal and ethmoidal sinuses were involved and contained the streptococcus. Cultures from the middle ear showed the diphtheria bacillus and the streptococcus.

In one of the four cases of uncomplicated scarlet fever the antra were found to be normal; in one, a double empyæma, the streptococcus and the staphylococcus pyogenes aureus and albus were found; in the third case the staphylococcus pyogenes aureus and albus, and the bacillus pyocyaneus were found; in the fourth case one antrum was involved which furnished cultures of the streptococcus and a short diplo-bacillus. Cultures of the streptococcus were obtained from the middle ear in both cases where there was infection on both sides.

*Conclusions.*—In fatal cases of diphtheria and scarlet fever, infection of the antra of Highmore is quite common. The micro-organisms commonly found are the diphtheria bacillus, the pus cocci, and the pneumococcus. In the cases reported the inflammatory changes in the antra did not produce sufficiently marked symptoms to attract attention during life. There is also, in many cases that recover, reason to believe that the accessory sinuses are often involved, some cases recovering without ill effects, in others subacute or chronic diseases

of these sinuses may be set up. The finding of diphtheria bacilli in the nose, even months after the membrane has disappeared, is probably best explained by antral involvement. Examination of the antral cavities would seem to be indicated in all cases where the diphtheria bacillus persists.

H. H. W.

**Preysing, H.** Die gesunde menschliche Paukenhöhle ist Keimfrei. Centrblt. f. Bakt., 25: 635-641, 1899.

After extensive bacteriological examination of the normal middle ear in man, the following conclusions were

drawn: That the normal middle ear is germ free. That the middle ear of the newborn, which is filled with mucous, is germ free. (In the latter the cases examined were few.) That serous exudates arising from a general dropsy are also free. That the cause of typhoid fever may also give rise to otitis media.

H. H. W.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

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### CURRENT LITERATURE.

**Homén.** Die Wirkung der Streptokokken und ihrer Toxine auf Verschiedene Organe des Körpers. (Arbeiten aus dem Pathologischen Institut zu Helsingfors.) Zeigler's Beiträge zur Path. Anat. 25: 1-272, Pl. X., 1899.

This work, by Homén and his pupils, occupies an entire number of the *Beiträge*, and is a study of the changes produced in the nervous system, liver, kidneys, heart, and peritoneal cavity.

Rabbits were used exclusively. The virulence of the streptococcus varied in different groups of experiments. The toxine was obtained in concentrated form from bouillon cultures by precipitation, either by ammonium sulphate or by amylic alcohol. Inoculations were made subcutaneously, intraperitoneally, in the ear vein, and into the trachea. The results of the work are stated briefly in the following:

**Homén and Laitinen.** Nervous System.

About three hundred animals were used. They were inoculated with the

streptococcus or its toxine in the right sciatic nerve, or in the spinal cord. The clinical symptoms varied; paralysis of the hind leg was frequent, and atrophy of the leg sometimes occurred in those which survived for a long time. Congestion and œdema of the sciatic nerve was seen after a week, in those inoculated with the streptococcus. The toxine produced very slight changes. Microscopically, the cocci are found to be diffusely scattered in the peripheral branches of the nerve bundle, and collected in groups in the lymphatics, beneath the perineurium in the proximal portion. They appear to reach the center of the nerve bundle along the endoneural terminations of the perineural sheath. In extending to the cord the cocci appear to travel along the dorsal nerve roots, after which they spread quickly through the intermeningeal tissues of cord and brain. Lesions produced by them are generally localized in the neighborhood of the anterior nerve roots. A week after injection no cocci can be found in the cord, but they may



be found as late as the seventeenth day in the injected nerve. Wherever the cocci become localized, there is a diffuse granular degeneration of the nerve elements, followed by immigration of leucocytes, and a proliferation of cells of the endoneurium and nerve sheaths. Later sclerotic changes occur. Degenerative changes are found in the ganglia of the dorsal nerve roots. The changes are practically the same whether the injection is made into the nerve or into the cord, and changes produced by the injection of the toxine are similar to those produced by the injection of the cocci. The writers believe that their investigations furnish an anatomical basis for the theory of an ascending neuritis.

**Silfvast.** Lung. Fifteen minutes after an intratracheal injection of the streptococcus, the epithelial cells of the lung are studded with cocci which they have taken up. The phagocytosis of these cells is more marked than that of the polymorphonuclear and large mononuclear leucocytes. No streptococci can be found in the lung after twelve days, but they are demonstrable in the bronchial lymph nodes up to the thirty-fifth day. The inflammatory changes produced by the cocci and by their toxine are similar, and are at first local and later diffuse. By the continued administration of toxine, the affected areas undergo connective tissue changes.

**Björkstén.** Liver. In all cases of general infection, irrespective of the method of inoculation, cocci are found in the capillaries of the liver, in all animals dying early, but not in those dying after the thirteenth day. The liver cells near these clumps of cocci are often changed. Sometimes necrosis, round cell infiltration, and hæmorrhages are found. After direct injection into the liver substance, the animal generally dies of acute peritonitis or of general infection. By injection through the common bile duct it is found possible to produce an infection of the liver, without marked general infection. In these cases the cocci are found in or about the bile passages where there is much small cell infiltration. Degeneration of the surrounding liver cells is seen; later there is an increase of connective tissue in these areas, leading finally to complete cirrhosis.

**Tallquist.** Heart. Direct injection of streptococcus into the heart muscle produces, in nearly all cases, a pericarditis fatal in from two to three days. In the heart muscle small abscesses develop in some cases. More often, however, there are small foci made up of necrotic muscle fibers, round cells, and cocci. Small hæmorrhages are frequent. Simple parenchymatous changes are seldom seen. After intravenous (ear vein) injections, changes in the muscles are quite constant. In addition to the changes produced by direct injections into the heart muscle, there is also seen, in some cases, hyalin degeneration of the muscle fibers, and proliferation of muscle nuclei. Interstitial changes are not uncommon in late cases. After intravenous injections of the toxine, granular, hyalin, and waxy degeneration of muscle fibers is seen. The interventricular septum and the wall of the left ventricle are the parts most commonly affected.

**Bornsdorff.** Kidney. The appearance of cocci in the urine is not constant. The shortest time was nine and a half hours after injection. In five cases, with a fatal termination after periods varying from seven to

fifty-five days, the urine was sterile throughout. In a few cases the cocci were present for a few days, and then disappeared. As a rule the number of micro-organisms eliminated increased with the course of the process. Microscopic examination of the kidney in early cases shows in the cortex desquamation of the tubular epithelium. In later cases there is slight fatty degeneration of the epithelium of the loops. Small cell infiltration and frank nephritis does not occur. Cocci are often seen in the glomerular tufts, less often in the inter-tubular capillaries, rarely within the tubules, and never in the capsular space.

**Wallgren.** Peritoneal Cavity. Bouillon cultures of streptococcus of varying virulence were injected, and fluid withdrawn after different intervals of time. Generally a fatal peritonitis resulted. The peritoneum can get rid of very small doses of virulent organisms with only a slight infection at the point of inoculation. Streptococci of slight virulence do not develop in the peritoneum if only a small amount of fluid is injected with them. The peritoneum is protected by its great power of absorption, the activity of the leucocytes normally in it and those which emigrate to it, and possibly by the activity of the endothelial cells. The leucocytes act by phagocytosis, by the products of their degeneration, and possibly also by their secretions. With very virulent streptococci there is marked phagocytosis at the beginning of the infection, later it is lacking; with organisms of slight virulence, the phagocytosis persists uninterruptedly as long as streptococci are present in the peritoneal cavity. As long as streptococci are present, and for two or three days after their disappearance, the majority of the cells are polymorphonuclear leucocytes. After this time the mononuclear leucocytes predominate.

R. M. P.

## NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA MCI. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

**Graetz, L.** Die Röntgenstrahlen in Beziehung auf Mineralogie und Krystallographie. Zeit. f. Kryst. **30**: 610-618, 1899.

The writer gives under five groups a brief statement of the work done and lists of the publications.

**I. Absorption of the Röntgen Rays.**—The permeability is dependent upon the density. In general, the denser substance absorbs most; that is, with elements and salts the absorption is greater as the molecular weight increases. Calcite absorbs more than its density warrants. Carbon and its combinations with oxygen, hydrogen, and nitrogen are relatively very transparent, while sulphur, phosphorus, and especially iodine, make substances relatively opaque. Phosphates absorb less than arsenates.

X-rays produced in very high vacuum are less absorbed than those from lower vacuum.

The precious stones ruby, sapphire, topaz, emerald, cats-eye are, in transparency, between diamond and quartz. Turquoise differs materially from its imitations, true pearls are more transparent than artificial, and in general the true stones differ notably in transparency from their imitations.

II. The Development of Fluorescence and Phosphorescence by the Röntgen Rays.—The X-rays produce luminescence in certain amorphous substances, such as uranium glass, and in crystals of certain salts and minerals. Fluorite becomes so strongly luminescent, that if added in fine powder to the photographic plate, the time of exposure for X-ray photographs may be shortened. Celestite, barite and strontianite produce similar effects. Diamond, apatite, autunite and scheelite become strongly luminescent, and many lead compounds feebly so.

III. Refraction of Röntgen Rays.—Prisms of water, carbon disulphide, and aluminum produce no refraction; with iron prisms there was very slight refraction, the index of refraction differing from unity not more than 0.0005. With a diamond prism the index of refraction differed from unity less than 0.0002, evidently within the limits of error in observation.

IV. Polarization and Dichroism of the Röntgen Rays.—The X-rays would be polarized if in different directions there is unusual absorption. No such difference was found in quartz, epidote or apatite, and in other minerals the results were negative.

V. Uranium and Thorium Rays.—Zinc sulphide and calcium sulphide produce an image on a photographic plate wrapped in black paper, that is, evolve rays which penetrate the paper. Uranium salts especially show the property. The rays from such substances penetrate most material even more easily than the X-rays.

A. J. M.

**Morozewicz, Jozef.** Experimentelle Untersuchungen über die Bildung der Minerale im Magma. *Tschermak's Min. u. Petr. Mitt.* 18: 1-94, 105-240, 1898.

This paper is a record of probably the most important work in mineral synthesis since the researches of Fouqué and Michel-Lévy\*. The experiments

extended practically without interruption through over five years, and differ from those of Fouqué and M-Lévy in that, instead of using small (20 cm. diam.) platinum crucibles in a laboratory furnace, large crucibles were inserted in one of the open-hearth furnaces of the glass works at Warschau. In this way much larger crystals were obtained, which could be separated and chemically analyzed, instead of relying solely upon examination with the microscope.

The furnace was in use throughout as a glass furnace, two openings (canals) were made in the walls one and one-half feet long, through which the crucibles could be inserted and withdrawn, and these were closed by suitable slabs. For melting, large crucibles were used, but after melting it was found most satisfactory to devitrify and crystallize in Chamotte crucibles of 150 cc. capacity.

The mixtures were prepared to correspond to analyses of unaltered, well crystallized, and typical eruptive rocks. For instance: for granite the mixture corresponded to the liparite of Iceland, for syenite to the trachyte of Ischia, and for basalt to the dolerite of Londorf. The bases were added as carbonates, hydrates or oxides, the silica as  $\text{SiO}_2 \cdot 3 \text{H}_2\text{O}$ .

\**Synthèse des Minéraux et des Roches.* Paris, 1882.

The charged crucible was first placed in one of the canals and brought to a red heat, then moved into the full heat of the furnace. When the charge was melted the crucible was moved back nearer the walls and left there for several days, and then moved into the still cooler canal.

The whole operation varied from one week to two months, and the temperature at the maximum heat of the furnace was about  $1600^{\circ}\text{C.}$ , and near the walls about  $1200^{\circ}$ , and perhaps  $600^{\circ}$  in the canals. During casting times these temperatures were very much lowered.

The conclusions of Lagorio† that a magma is a solution of different but definite silicates in undetermined proportions and that these solutions in crystallizing are governed by the same laws as an aqueous solution of several salts, were taken as the basis of the investigation.

*Conditions governing the formation of Corundum, Spinel, Sillimanite, and Cordierite in Silicate Magmas.*—The result of a large number of meltings under different conditions, and of a careful chemical and optical analysis of the products, lead to the following very definite conclusions.

After defining silicate magmas saturated with alumina as those in which the ratio of other bases to the alumina is unity, the conclusions are, in brief :

1. That such saturated solutions can at higher temperature dissolve alumina and become supersaturated.

2. That soda alumina silicates dissolve alumina in large amounts, lime (or magnesia) alumina silicates dissolve alumina in small amounts, and pure potash alumina silicates do not dissolve alumina at all.

3. The formula of a silicate magma supersaturated with alumina may be written:  $\text{MeO}, m \text{Al}_2\text{O}_3, n \text{SiO}_2$ , in which  $\text{Me} = \text{Na}_2, \text{Ca}, \text{K}_2$ , etc.

Upon crystallization of such a magma the *entire excess* of alumina ( $m-1$  parts) separates in the form of: A. Magmas with less than  $\frac{1}{2}$  per cent. of  $\text{MgO} + \text{FeO}$  :

(a) Corundum if  $n$  is less than 6.

(b) Sillimanite, or sillimanite and corundum, if  $n$  is greater than 6.

B. Magmas with over  $\frac{1}{2}$  per cent.  $\text{MgO} + \text{FeO}$  :

(c) Spinel, or spinel and corundum, if  $n$  is less than 6.

(d) Cordierite, or spinel and cordierite, if  $n$  is greater than 6.

4. The amount of spinel, corundum, or sillimanite depends upon and is strictly proportionate to the degree of supersaturation with alumina.

5. The crystallization does not depend upon the basicity of the magma, for  $n$  may vary from 1 to 13, but only upon the ratio of the other bases to the alumina, and separation can take place whenever there is more than one part of alumina to one part of other bases.

Evidently the sequence of crystallization is not based upon fusibility, but upon supersaturation ; crystals of a substance can only separate from a solution supersaturated with the substance.

As a result of examination of pyroxene crystals obtained, the author concludes : The pyroxenes separated from silicate magmas, are isomorphous mixtures of Me

† Ueber die Natur der Glasbasis, sowie der Krystallisationsvorgänge im Eruptiven Magma.



$\text{SiO}_3$ ,  $\text{MeR}_2\text{SiO}_6$ , and  $\text{Me}_2\text{R}_2\text{Si}_4\text{O}_{12}$  ( $\text{Me} = \text{Ca, Mg, Fe Na}_2$ ; [ $\text{R}_2 = \text{Al}_2 \text{ Fe}_2$ ]). The  $\text{MeR}_2\text{SiO}_6$  can form independently apparently as a rhombic pyroxene. In acid magmas the  $\text{MeSiO}_3$  preferably unites with the  $\text{M}_2\text{R}_2\text{Si}_4\text{O}_{12}$ , but in more basic with  $\text{Me}_2\text{R}_2\text{SiO}_6$ . In magmas containing about 50 per cent.  $\text{SiO}_2$ , rhombic pyroxene forms if the ratio of  $\text{MgO} + \text{FeO}$  to  $\text{CaO}$  is equal to or greater than 3, if the ratio is notably less, monoclinic pyroxene separates.

Over 30 species are described, the methods of examination detailed, and photo-cuts given of the crystals obtained.

A. J. M.

Von Federow, E. Ueber Krystallzeichnen. Zeit. f. Kryst. 30: 9, 1898.

This article is designed to supplement Goldschmidt's method\* of construction of clinographic projections from the gnomonic projection without using crystal axes.

A. J. M.

Von Federow, E. Ueber Isomorphismus. Zeit. f. Kryst. 30: 17-22, 1898.

**Diamond Saw (Improved Pattern).** Made by Chas. L. Whittle, 7 Exchange Place, Boston, Mass. *Catalogue circular.*

Saws 6 to 10 inches in diameter can be adjusted to any ordinary lathe, and complete directions are given for use and speed of rotation. A 6-inch diameter saw, properly handled, should cut 300 to 400 square inches of average rock and much more of limestone, etc. Price: 6 in. saw, \$5.00; 12 in. saw, \$10.00.

L. McL. L.

**Petrotome or Rock Slicing Machine.** Invented by Wm. B. Dwight, and made by C. H. Cowdrey Machine Works, 5 Main st., Fitchburg, Mass. *Catalogue circular.*

Cuts unusually large planes (2 to 4 sq. inches) of sections in any required direction, or series of parallel planes. By special accessory apparatus a series of parallel planes can be cut in a single operation. Trims large cabinet specimens very conveniently. Detailed description and cut given. Price, \$200.00.

L. McL. L.

#### INDIVIDUAL SPECIES.

**Augelite** from a new locality in Bolivia. L. J. Spencer. Min. Mag. 12: 1, 1898.

Occurs sparingly in veta (vein) carmen of the silver mines at Tatasi and Portu-galete, Sud-Chicas, Dept. Potosi. Colorless and transparent, showing usual perfect cleavages, and generally imbedded in massive pyrite. Closely resembles barite. Forms of observed crystals given. Indices of refraction for Na light, 1.5752 and 1.5893.  $2E = 82\frac{1}{2}^\circ$ .  $G. = 2.69$ .

L. McL. L.

**Argyrodite** (stanniferous) from Bolivia: The identity of the so-called "Crystallized Brongniardite" with Argyrodite-Canfieldite. G. T. Prior and L. J. Spencer. Min. Mag. 12: 5, 1898.

Found at Aullagas, Chayanta, Dept. Potosi, as small, dull black, isometric crystals encrusting pyrrargyrite. Interesting crystallographically on account of the twinning, and chemically in giving a different ratio of Sn and Ge from that found in original canfieldite. Composition = 5 ( $4 \text{ Ag}_2 \text{ S. Ge S}_2$ ) + 2 ( $4 \text{ Ag}_2 \text{ S. Sn S}_2$ ).  $G. = 6.19$  at  $18^\circ \text{ C}$ .

Also found to be identical with the cubical crystals referred by Damour to brongniardite (proved by chemical and physical re-examination of the latter mineral).

The occurrence of tin ores in Bolivia (according to Stelzner) is peculiar,

\*Zeit. f. Kryst. 19: 352, 1891.

being found with metallic sulphides in ordinary mineral veins in trachytic and andesitic rocks, and not as usual in deep-seated granitic rocks. L. McI. L.

**Diamond**, Artificial Production of, in Silicates corresponding to the Actual Mode of Occurrence in South Africa. I. Friedländer. Geol. Mag., 1898.

Undoubted microscopic diamonds obtained, under normal pressure, by fusing with a blow-pipe a small piece of olivine and stirring with a little rod of graphite.

Author infers that the South African diamonds may have been formed in a similar way, by the action of a molten silicate (as olivine) on graphite. In that locality carbonaceous shales are interrupted by the diamond-bearing rock. Not probable that the diamonds were formed in molten iron at great depths under great pressure (conditions used by M. Moissan in making artificial diamonds) and then floated into the molten silicate rock above. L. McI. L.

**Notes** on Anthophyllite, Enstatite and Beryl (Emerald) from North Carolina. J. H. Pratt. Am. Jour. Sci., 4, 5: 429, 1898.

*Anthophyllite* occurs at Bakersville, Mitchell county, in boulders of altered dunite, as seamed and cracked prismatic crystals of clove-brown to flesh-red color, and imbedded in penninite. Found also at Corundum Hill, but more fibrous and inferior in quality.

*Enstatite* found at Corundum Hill, forming a rock of interlocking grayish crystals. Bronzite occurs in outcrop of dunite at Webster, Jackson county.

*Beryl* (emerald) occurs in a pegmatite vein in midst of gneiss and biotite schist, on divide between Brush and Crabtree creeks, Mitchell county.

L. McI. L.

**Orthoclase** as gangue mineral in a fissure vein. W. Lindgren. Am. Jour. Sci., 4, 5: 418, 1898.

Formed by aqueous deposition, but not common. Usually the variety adularia. The very common presence of

carbonic acid, in thermal waters, probably prevents the abundant formation of gangue orthoclase, as the more stable compounds, muscovite, or sericite would be formed.

L. McI. L.

**Whewellite vom Venustiefbau bei Brück.** Schuert, Richard. Tschermak's Min. u. Petr. Mitt. 17: 251, 1898.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace, Biological Laboratory, University of Rochester, Rochester, N. Y.

### REVIEWS.

**Monckeberg, Georg, and Bethe, Albrecht.** Die Degeneration der markhaltigen Nervenfasern der Wirbelthiere unter hauptsächlich Berücksichtigung des Verhaltens der Primitivfibrillen. Archiv. f. Mikr. Anat., 54: 135-184, pls. 8-9, 1899.

cutting or by pressure, and material was fixed in .25 per cent. osmic acid solution for twenty-four hours.

The nerve was studied under normal and pathological conditions with especial reference to the rôle of the primitive fibrils in degeneration. Dogs and frogs were used, lesion was caused by

The normal nerve consists of primitive fibrils imbedded in a perifibrillar matrix and enveloped in three sheaths, the sheath of Schwann, the medullary sheath, and an inner sheath. Monckeberg admits the possible granular structure of the fibrils, but considers that the granules are more in the nature of molecules and not visible. The fibrils are of the same thickness throughout and are free from varicosities. The sheath of Schwann, the medullary sheath, and the perifibrillar substance are broken into segments at the nodes of Ranvier, but the fibrils are continuous from the centrum to the periphery. This is taken as evidence that the primitive fibrils form the conducting substance.

A delicate line may be seen between contiguous segments of the nerve, giving the impression that the substance of the two segments is separated by a capillary space, and a precipitate is formed here in material fixed with osmic acid, or silver solution.

The study of the degenerating nerve showed that the fibril consists of two parts, an insoluble substratum, and a substance in chemical or mechanical union with this which is easily soluble in alkalies and reacts to basic stains. When this stainable substance is gone the nerve loses its irritability. Degeneration depends upon changes set up in the primitive fibrils, and going hand in hand with changes in the matrix. The fibrils form spindle-shaped thickenings and break up into coarse granules, which fragment and are resorbed. Degeneration begins at the point of lesion, and is total toward the periphery, but partial toward the centrum. The rate of degeneration varies in summer and winter frogs. Sensory fibers degenerate more rapidly than motor fibers, degeneration is more rapid in thin than in thick fibers, and it varies in different parts of the same fiber. There does not appear to be sufficient proof of a difference between traumatic and secondary degeneration.

E. M. B.

**Abraham, Dr.** Die Durchschneidung des Nervus Mandibularis. *Archiv. f. Mikr. Anat.* 54: 224-254, pl. XII, with 8 text-figs., 1899.

This is an investigation of the question which has been under discussion for the last fifty years as to the existence of trophic nerve fibers. Experiments were made upon kittens, and upon about forty dogs ranging in age from eight days to three or four months. The teeth were chosen for study, partly from the writer's familiarity with that tissue, and partly because the tissue, being highly differentiated, does not possess a variety of functions which would complicate the investigation of a single series of changes that might be set up. The innervation of certain teeth was destroyed by cutting the mandibular nerve of the left side, and to prevent regeneration, as much of the nerve as could be reached—a half centimeter at least—was extirpated. The right side was left intact for comparison. The wounds healed readily, and later both left and right sides of the under jaw were removed and placed in picric-acetic-sublimate, decalcified with three per cent. nitric acid, imbedded in celloidin, and stained after the Van Gieson method. In the forty cases there were no histological differences between the teeth of the right and left sides except in one instance, where the slight changes presumably resulted from injury to the artery supplying the tooth. A quantitative chemical analysis of teeth from each side showed 20.6 per cent. of organic matter and 56.58 per cent. of lime in those from the right side and 19.3 per cent. organic

matter with 56.77 per cent. of lime in those from the side that had been operated upon. Experiments were also made upon kittens to determine the effect of innervation upon the regeneration of the mucus membrane of the lip. Resection of the mandibular nerve in kittens from one to two weeks old had very slight effects or none at all, but produced changes in kittens that were several months old. This is thought to be due to the fact that the younger kittens feed by sucking motions, while the older ones have energetic chewing motions. The experiments show that the growth of the teeth is independent of nervous influence and that the mandibular nerve does not contain fibers which affect nutrition. It appears that certain tissues and organs may grow and be regenerated without the active influence of the nerves.

E. M. B.

**Thomson, Dr. H. Campbell.** On the Structure of Brain Cells and their Degeneration in General Diseases. *Lancet*, **I**: 1428-1429, 1899.

This describes histological changes in the brain cells of certain patients. Fresh tissue was stained with methylen blue, care being taken to guard against post-mortem changes. Normal cells showed an even distribution of chromophile granules. Cells from two cases of meningitis in children, respectively fourteen months and one year of age, showed the cells stained in patches of different intensity, no differentiation of chromatophile rods, the nucleus absent or ill-defined and the nucleolus sometimes displaced. Cells from a case of septicæmia in a child of five years presented a dark blue appearance with no differentiation. The nucleus was not well defined, but the nucleolus as a rule was unchanged. Cells from a case of tetanus showed loss of chromatic substance and presented an appearance of sponge work on a white background. The meshes of the sponge work were stained brown by the methylen blue.

E. M. B.

**Turner, Aldren, and Hunter, William.** On a Form of Nerve Termination in the Central Nervous System, Demonstrated by Methylen Blue. *Brain*, **22**: 123-136, 1899.

The results obtained are in accord with the neuron theory, and support the view that impulses are transmitted by the contact of neural processes. The point of difference from the observations of others consists in the finding of a basket-like network into which the cellulipetal nerve fiber breaks up at its termination. This network is disposed over the surface and the base of the protoplasmic processes of the cell with which it comes in contact. From this it is assumed that the impulses are transmitted from the terminal directly to the cell body. The network was found only by means of methylen blue staining and is not comparable with the basket-work of Purkinje cells. Observations were made on monkeys, cats, dogs, rabbits, and mice, and terminations of this kind were found in the cerebral cortex, optic thalamus, and various nuclei of the brain.

E. M. B.

#### RECENT LITERATURE.

**Ruffini, A.** Sulla fina anatomia dei fusi neuromuscolari del Gatto e sul loro significato fisiologico. pp. 50, 2 tavole. Siena, 1898.

**Acquisto, V.** Sulla struttura delle cellule nervose nei gangli, spinali dell' uomo. *Monit. Zool. Ital.*, pp. 49.

**Thilo, O.** Die Augen der Thiere. 24 pp., 2 pls. Hamburg, 1899.

**Hohne, O.** Beiträge zur Kenntniss des Tastsinnes der Haut und der Schleimhäute, besonders in der Medianlinie des Körpers. pp. 33, 2 cuts. Rostock, 1898.

**Donaldson, H. H.** A Note on the Significance of the Small Volume of the Nerve Cell Bodies in the Cerebral Cortex of Man. *Jour. Comp. Neur.*, **9**: 141-149, 1899.



## Received for the Journal Library.

(All publications received for this library are carefully filed and preserved for reference.)

The JOURNAL acknowledges the receipt of the following publications:

- Annual Report of the Smithsonian Institution.** U. S. National Museum. 1107 pages, with illustrations, 1896.
- Explorations in the Far North.** Frank Russell. pp. 288, a map and illustrations, 1898.
- Papers and Addresses.** N. Y. State Veterinary College. With plates and figures, 1896-1898.
- Bulletin of the Illinois State Laboratory of Natural History.** Vol. V.
- ARTICLE I.** Plankton Studies. Methods and Apparatus in Use in Plankton Investigations at the Biological Experiment Station of the University of Illinois. C. A. Kofoed, Ph. D. 7 plates, 1 text-fig., pp. 1-24, 1897. This gives methods for taking hauls, descriptions of nets and apparatus, and methods for the preservation and examination of plankton.
- ARTICLE II.** A Contribution to a Knowledge of North American Fresh-water Cyclopidae. Ernest Forbes, B. S. Bibliography, 13 plates, pp. 27-82, 1897. A comparative study of American and European species of cyclops, with the purpose of revising the nomenclature, and determining characters for specific distinction.
- ARTICLE III.** The North American Species of Diaptomus. Frederick William Schacht, B. S. Bibliography, 15 plates, pp. 97-207, 1897.
- ARTICLE IV.** The North American Centropagidae belonging to the Genera *Osphranticum*, *Linnocalanus*, and *Epischura*. Id., pp. 225-269, 1898.
- ARTICLE VI.** A List of the Protozoa and Rotifera found in the Illinois River and Adjacent Lakes at Havana, Ill. Adolph Hempel, M. S. 5 text-figs., pp. 301-388, 1898. Methods are given for obtaining and preserving protozoa and rotifera, with descriptions, and a classification of species.
- Botany at the Anniversary Meeting of the American Association for the Advancement of Science.** Dr. Erwin F. Smith, secretary. Science, N. S. VIII, pp. 651-660, 690-700, 1898.
- Transactions of Vassar Brothers Institute and its Scientific Section.** Vol. VII, 1894-1896.
- Two New Species of Orthozia.** J. D. Tinsley. 2 text-figs.
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- On a New Apochromatic Objective Constructed without the Use of Fluorite.** Philip E. Bertrand Jourdain, F. R. M. S.
- On a Method of Adjusting the Sizes of the Colored Images Yielded by the Cook Lens.** Id.
- Remarks on the Construction of the Planar Lens and its Use in Low-power Photomicrography.** Id., pp. 395-400, 3 text-figs. Jour. R. Micr. Soc., 1898.
- Die Ursachen der Braun oder Schwartz-Trocken-Faule des Kohls.** Dr. Erwin F. Smith. Zeitschrift f. Pflanzenkrankheiten. VIII Bd., III Heft, pp. 1-4. 1 pl.
- Acute Chloral Dementia Simulating Paretic Dementia.** Henry Waldo Coe, M. D. Medicine. pp. 1-3, August 1898.
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- Notes on the Flora of Northeastern Iowa.** Id. Iowa Academy of Sciences, pp. 1-69, 1897.
- Notes on the Country Rock of the Kalgoorlie Gold Field, Western Australia.** George W. Card, A. R. S. M., F. G. S. Records Geol. Survey, N. S. Wales. Vol. VI, pp. 17-42, pl. I-III, 1898. An account of the geologic history of this region, based on microscopic, chemical, and structural characteristics.
- A Method of Preparing Test Objects for Disinfection Experiments.** Dr. Hibbert Winslow Hill. Trans. Am. Pub. Health Ass'n. XXIV, pp. 1-4, 1 pl., 1898.
- Heater for Glycerine Jelly Slides.** J. Franklin Collins. Bryologist II, pp. 21-23, 1 fig., 1899.
- Early Diagnosis in Whooping Cough.** H. L. Wagner, M. D., Ph. D. N. Y. Med. Jour., Oct. 8, 1898.
- Oedematous Changes in the Epithelium of the Cornea, in a Case of Uveitis Following Gonorrhoeal Ophthalmia.** Edward Stieren, M. D. Johns Hopkins Med. Bull., No. 93, pp. 1-6, 1 pl., 1898.
- The Gonococcus.** William C. Mitchell, M. D. Med. News, pp. 1-10, 1 text-fig., Dec. 3, 1898.
- Proceedings of the Linnean Society of New South Wales.** July 28, 1898.
- The Value of Surgery in Nervous Diseases.** Henry Waldo Coe, M. D. West. Med. Rev., pp. 1-14, June, 1898.

# Journal of Applied Microscopy.

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## Botany in Secondary Schools.

Formerly there was but one way to teach botany, for a single phase of it only was available. The gross structures of flowering plants were examined and their names memorized, and the study culminated in "analysis," which by a strange misapplication had come to mean the discovery of the names of plants. With increasing knowledge this method of teaching came to seem very partial and superficial. It must be confessed, however, that many schools have not outgrown this primitive stage.

A second period of botanical instruction was ushered in by the equipment of laboratories with compound microscopes. Plants of the lower groups now came into notice, and some knowledge of the general "make-up" of the plant kingdom was developed. At first the pendulum swung to the opposite extreme, and the contact was chiefly with the lower plants and through the microscope. The student familiarized with flowering plants out-of-doors became the student familiarized with structures under the microscope. Probably this is the favorite method of instruction in the better equipped schools to-day.

This method has been criticised as leading to no knowledge of plants as they exist in nature. The laboratory student, transferred to forests and fields, seemed to have no more knowledge of his surroundings than he had before studying botany. It was questionable whether he could recognize in place the material he had seen only in the laboratory. Another important criticism was that students whose formal training ends with the secondary school cannot carry such a contact with plants into their subsequent experience, and botany becomes a closed subject rather than one full of perennial interest.

A third method is coming into recognition, which seeks to combine the advantages of the other two, and also introduces an entirely new standpoint. The most conspicuous fact in reference to a plant is that it is alive and at work. In order to do this work, the organs must be related properly to their surroundings, and the study of these life-relations seems to be the natural approach to plant structures.

For example, the life-relation of a foliage leaf is its relation to light, and this explains its position, its form, its relation to other leaves, and also its structure. In the presence of this great fact the names of forms and positions of leaves become matters of very small importance, and there is no danger of mistaking definitions of words for knowledge of things. Again, the life-relations of roots

are various, as relations to soil, to water, to air, to mechanical support, etc. Perhaps flowers and their clustering have been more deeply buried beneath terminology than any other plant structures, but all of this is brushed aside when the life-relations are found to be pollination and seed-distribution.

This new standpoint does not abandon the microscope and the structures which it reveals, but it approaches them with a new purpose. Nothing can replace a general acquaintance with plant forms, for they must be known in order to illustrate the various methods of work. Nothing is simpler than to devise experiments in the laboratory to illustrate the common life-relations, and to supplement the experiments with observations in the field.

It is found further that plants are not scattered in hap-hazard fashion over the surface of the earth, but are organized into definite associations or societies. A certain combination of water, soil, heat, etc., determines a plant society, and in it certain plants are permitted and others forbidden. The study of these plant associations, the conditions which determine them, and the adaptations of the plants, is most interesting and important. School plantations can reproduce many conditions and plant associations, so that much of such study may be carried on in the laboratory.

For example, aquaria or glass jars can be used to contain representative forms of the common societies of water plants, as floating forms, swamp forms, etc., and their characteristic adaptations in position and structure studied. In boxes may be grown representatives of dry sandy ground societies, also societies of the more fertile soils, etc. To contrast these societies and their adaptations is full of profitable work.

Such a view of plants is a permanent possession, for every landscape becomes significant, and in the student's subsequent experience such material is constantly presenting itself. It is such a background that the universities desire for their botanical work, for the forms studied in their laboratories are thus put into their proper places in nature through previous experience.

The secondary schools can not introduce such work in revolutionary fashion, but must gradually intersperse it among the old work as training and familiarity with the material may permit. Those accustomed to an older method, and adjusted to its demands, in the presence of the new may feel the shrinking which comes from inertia, but this paper has in mind, not the feeling of the teacher, but the good of the pupil.

JOHN M. COULTER.

Head Professor of Botany, University of Chicago.

## The Microscope, its Educational and Practical Value.

The saying that "truth is stranger than fiction" nowhere receives more striking confirmation than in the revelations of the microscope. By its aid the most commonplace and even insignificant objects are shown to be possessed of beauty unapproachable by human device. Indeed it is a curious fact that the more we magnify human productions the greater their imperfections appear, while nature's handiwork requires a microscope to show its greatest perfections.

It has been truly said that the microscope is our "sixth sense." It is our "open sesame" to worlds innumerable.

The microscopist has no need to sweep the heavens to discover new worlds; they are literally everywhere. Nor need he ever sigh for more worlds to conquer, for no branch of science—and science is only systematized observation—has reached that degree of perfection where there is no round still further up the ladder of knowledge. Indeed, we have not even yet progressed so far as to know what there is to learn, to say nothing of entering and possessing the land.

There are those who claim that all who use the microscope should devote themselves to some special line of study; in short, be specialists. Considering, however, the universal utility and pleasure to be derived from the microscope, this claim is very unjust. For the many, the microscope is an open book, or rather, a whole library of open books.

A microscope will suggest more queries in five minutes than can be answered in a lifetime.

We owe more to the microscope than we are conscious of, and to the physician most of all this fact should be thoroughly cognizant. The importance of the microscope in medical practice is yet in its infancy; in chemical analysis, in the study of minute anatomy, in the detection of physiological functions, in pathological examinations, and in the determination of the origin and causation of disease, the aid of the microscope is invaluable. The great germ theories of disease have been so systematically and thoroughly advanced, that many of them have already ceased to be theories, but are now established facts.

The physician, druggist, and chemist who does not use the microscope may well question whether he is doing justice to himself, to say nothing of justice to his patrons.

The clergyman, if he would keep abreast with the times, must be familiar with the revelations of the microscope. The structure of the humblest insect, which we ruthlessly crush, abounds in sermons.

To the perplexed and harassed man of business this fascinating form of varied communion with soothing nature, which the microscope yields, may afford just that ounce of prevention which will prove his salvation from the nervous prostration so common in long-strained mental activity.

The use of the microscope will soon be obligatory in the public schools, and the teacher who acquaints himself with its manipulations in advance, not only adds to his usefulness, but stands on a vantage ground over his less enterprising competitors.

To ladies, with their love of the beautiful, their abundant leisure, their quick intuitive perceptions, and their nimble fingers, the use of the microscope is particularly commended.

In the family circle, also, the microscope is invaluable; strengthening home ties, furnishing an interest ever fresh, in which all, both young and old, may join.

The popularity of the microscope is rapidly increasing, and very soon this ingenious instrument will be as necessary an adjunct for the completion of a household as a piano or Webster's dictionary.

Its value to the medical man is already recognized and appreciated by the



profession; but a few years will suffice to place the physician, who does not understand its manipulations and advantages in the rear ranks in the onward march of progress and success.

The time is not far distant when the microscope will be an indispensable adjunct to the advancement of every art and science, to the professional and to the layman, recognized as one of the most potent of civilizing factors.

M. A. GOLDSTEIN, B. S., M. D.

St. Louis, Mo.

## Notes on the Histology of the Amnion.

I recently utilized for microscopical purposes a human afterbirth, delivered at full term in a normal labor, which possessed two distinct fetal sacs, the amnion being completely separate from the chorion and never having become united to the latter, as is ordinarily the case. The anomaly was reported in the *Medical News*, New York, July 1, 1899, page 12. The thin and distinct amnion in this case, with its free and definite outer surface, presented such distinct pictures of the outlines of the mature amnionic cells, as brought out by the silver-nitrate method, in conjunction with nuclear stains, that a note of the appearances presented seems worthy of record.



Figure 1. Epithelial cells lining inner surface of the amnion. (x 1000.)

1. The epiblastic cells lining the free inner surface of the amnion (that directed toward the fetus) were simple squamous epithelium cells (Fig. 1). They were flat and thin, uniting in a single layer edge to edge, with slightly wavy margins, and were polygonal (often pentagonal and hexagonal), elongated, or irregular in shape. Their nuclei were circular or oval; most of the cells were uninucleated, but cells containing two, three, or even four nuclei were common. In size the dimensions of the cells (diameters or diagonals) ranged usually from about .015 to .022 millimeter, the extremes measured being .011 and .033 mm.; the nuclei were about .004 to .0055 mm. in diameter. Similar appearances and results as to these epithelium cells were obtained in silver-nitrate preparations of the membranes from a normal afterbirth.

2. The subepithelial or outer portion of the amnion, making up the chief thickness of the membrane, is a mesoblastic connective-tissue layer, consisting

of large numbers of connective-tissue cells imbedded in delicate intercellular material. These connective-tissue cells, as observed, were mostly large, irregular, thin, flat cells, arranged flatwise with the surface, and occupying the middle plane of the amnion. These cells were very irregular in form, giving off irregular branches and processes, some broad, some fine and filamentary. The processes of neighboring cells often were directly continuous with one another. The general outlines of the smaller of these cells were often rounded, of the larger roughly polygonal, or altogether irregular. In size these cells ranged from .025 to .090 or even .100 mm. in extreme dimensions, measuring between the

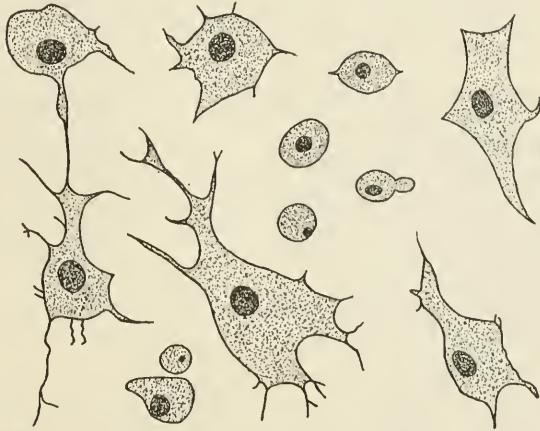


Figure 2. Connective-tissue cells in the amnion. (x 500.)

extremities of the processes (see Fig. 2). Mingled with these large, irregular cells, were smaller cells of rounded or oval outline, ranging from about .008 to .016 mm. in diameter. Some of these cells had one or two fine processes, others had none (Fig. 2). In one place, in one of the specimens, a capillary tube was traced for a short distance in the connective-tissue layer, and in its vicinity were a few scattered leucocytes.

3. In places, the outer surface of the amnion (that directed toward the chorion, and in this case a definite free surface) was lined with a single layer of small flat cells, apparently of an endothelioid nature (see Fig. 3). These cells were mostly hexagonal in shape (some pentagonal and heptagonal), quite regular and uniform in size and shape; their angles were slightly rounded. They were united to one another by their edges or sides, which were straight, not sinuous. They were of small size, ranging from about .0055 to .007 mm. in diameter. They did not form a complete lining over the entire outer surface of the amnion, or at least the silver nitrate showed their boundaries only in patches. No nuclei appeared in them; if present they did not take the nuclear stains employed. At one point three or four of the cells seemed to exhibit relatively large single or double nuclei; but these could

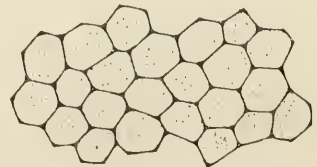


Figure 3. Endothelioid cells of outer surface of the amnion (and inner surface of the chorion). (x 1000.)

not be indubitably distinguished as belonging to the endothelioid cells rather than to some leucocytes which were immediately underneath.

Patches of cells precisely similar to these small endothelioid cells were also observed on the innermost surface of the chorion in this case (which was a definite free surface and separated from the amnion by a space). In the membranes from a normal afterbirth, in which the amnion and chorion were grown together in the ordinary manner, similar cells were observed at a plane corresponding to the deepest part of the amnion or innermost part of the chorion. The accompanying figures are from actual sketches made from the microscope.

J. B. NICHOLS, M. D.

Medical Department, Columbia University, Washington, D. C.

## Cutting and Mounting Sections of Cereal Grains.

While sections of all other conceivable parts of plants, from root to stem, branch, bud, and fruit, are commonly found in most collections of slides among botanists and microscopists, it is not a little remarkable that permanent mounts of such great economic and educational importance as sections of wheat and other grains, are never seen; at least the writer, during more than twenty years association with botanists and microscopists, has never seen one.

Undoubtedly the extreme rarity of such preparations is caused by the difficulty of making a presentable mount, or one that is tolerably free from the cloud of starch, that persistently flows into the surrounding mounting medium, in attempting to place the section under a cover-glass.

Having undertaken to prepare such a section for the teacher of the cooking class, that she might be enabled to elucidate more fully to her pupils the explanation she was making, regarding the relative nutritive values of different qualities of flour as dependent on various methods of milling, I soon discovered why sections of grain are not seen in the boxes of the Micro-Postal Club, and are not for sale at the opticians.

After spending many hours in unsuccessful attempts, however, I finally reached a fairly successful result, and the great interest manifested by both teachers and pupils upon examining these sections under the microscope, not only fully repaid me for all the labor and perplexities involved in their preparation, but illustrated in a striking manner the value of the microscope as an educator in our public schools.

For the purpose of saving teachers, and others who may wish to prepare such sections, a similar expenditure of time, I give here the steps I finally adopted in their preparation.

No especial difficulty is encountered, and little experience is required in making satisfactory sections of grains.

The first step is to soften the kernels slightly, and not too much, by immersion in water. If too soft the starch will fall out of the cells in cutting, and if too hard the sections will crumble before the knife. Wheat will be sufficiently softened if kept moist four or five hours, rye five or six, barley ten or twelve, and oats not more than one or two hours.

Imbedding may be done in any convenient manner, as the grains are easily

cut when slightly softened. Paraffin is perhaps the best material for the purpose, as it holds the grain so firmly that it may be cut in any direction. No elaborate microtome is necessary, any simple section-cutting contrivance answers quite well. Great care must be exercised, however, to have a knife with an extremely thin and sharp edge. The upper surface of the knife must be wet with alcohol, and the sections are best removed with a camel's hair pencil, and if it is desired to keep them for any length of time, they may be placed in a shallow dish of alcohol, to which is fitted a ground-glass cover.

The requisite degree of thickness, or rather of thinness, is a matter of some importance, for if too thin the starch grains fall out in cutting, and the gluten cells are disagreeably opaque if too thick. But as a great number of sections may be readily cut, a selection of such as are entire, and of a sufficient degree of transparency may be made with a hand lens.

The sections cannot be mounted in balsam, as that renders the starch grains so transparent that they are quite obliterated under the microscope, but glycerine jelly is a very satisfactory medium, and is soon made ready by placing the containing bottle in a cup of hot water.

Having slides and cover-glasses in readiness, take a section on a camel's hair pencil and place it upon a slide, the center of which has been marked on the back by a dot of ink.

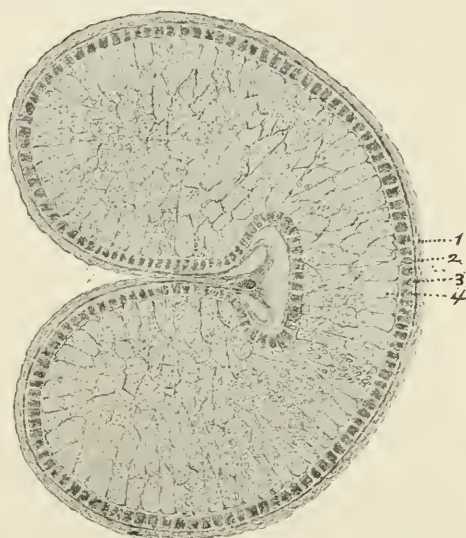
If the slide is held inclined, a little water, placed with the brush above the section, will run down and carry with it nearly all the surrounding grains of starch, but if any remain they may be readily removed with the brush.

After absorbing all surrounding water, place a drop of glycerine jelly upon the section, take a cover-glass in the forceps, moisten one side with the breath, and placing its center over the section, lower it carefully upon the gelatin, and if this is sufficiently fluid, the cover will settle to its place without using any pressure.

Any attempt to move the cover, or to press it down after it is in place, will cause a cloud of starch to issue from the cells.

Should the gelatin become so hard that the cover-glass does not settle down of its own weight, warm the slide gently over the lamp.

When the section is mounted and placed under the microscope, we have the appearance presented in the drawing, which represents a grain of wheat amplified about forty diameters.



Transverse Section of a Grain of Wheat.  
Variety, Buda Pesth,  $\times 20$ .  
1—Fruit coats; epidermis, mesocarp, and endocarp. 2—Seed coats. 3—Gluten cells, or perisperm. 4—Starch cells, or endosperm.



The extreme outer coat (1) is the epiderm or husk, and constitutes what in milling is called bran. It consists essentially of cellulose and contains no important nutritious element. The next inner layer (2), quite thin, contains some oil and albumen.

The rectangular, symmetrically arranged cells (3) are filled with gluten, and as these cells follow the contour of the grain and extend around the deep furrow on the side, they constitute no inconsiderable portion of the entire kernel, the gluten, in different varieties of wheat, amounting to from ten to twenty per cent.

The entire interior (4) is filled with cells of irregular shape containing starch, the cell walls themselves are extremely thin, and have an element of gluten in their composition.

The finest and whitest wheat flour consists, of course, almost entirely of starch, the more nutritious, nitrogenous, elements in the gluten being excluded in the process of milling.

In sections of wheat, rye, oats, and Indian corn, only a single row of gluten cells is seen, while in barley there are three, but the cells being much smaller, the quantity of gluten contained is not so greatly in excess as the number might indicate.

The teachers of cooking employed in our public schools are now required to possess not only a simple knowledge of preparing palatable dishes, but must bring to their profession such scientific attainments as enable them to instruct their pupils in the nutritive value of various articles of food.

These teachers now uniformly recommend and teach their pupils to make bread and biscuits of flour from what is called the whole wheat, which contains the entire gluten element of the grain, the indigestible epidermal coat only being removed in grinding.

Bread made from this flour, although darker in color and requiring a somewhat different process in its preparation, is very sweet and palatable, and possessing all the chemical elements of a complete food, is undoubtedly destined, in the near future, to replace the white, starchy bread now in general use.

J. D. HYATT.

Principal of Public School No. 85, New York City.

## Filling Fermentation Tubes.

Formerly I was much annoyed by reason of the sugar bouillon boiling over during sterilization, wetting the cotton plugs, and leaving a large air bubble in the closed end of the tube. I concluded that the air contained in the bouillon was responsible for the trouble, and tried filling the tubes with *hot* bouillon only, from which the air had been thoroughly expelled by a stay of some minutes in the sterilizer immediately before use. This simple expedient practically eliminates the trouble from wetting of the plugs, and the air bubble which remains in the closed arm of the tube after sterilization is so small that it is readily absorbed as the tubes cool.

W W. ALLEGER.

## A Differential Stain for Goblet Cells in the Small Intestine.

The small intestine of the cat injected with the ordinary gelatin injection mass, hardened in 70 per cent. alcohol and imbedded in celloidin in the usual way, was cut in sections seven to fifteen microns in thickness. The sections were first stained in Mayer's hæmatein fifteen to thirty minutes, washed in 70 per cent. alcohol, then stained separately on the slide in a solution of Bismark brown in 70 per cent. alcohol for a very short time. The mucus-containing goblet-cells are stained brilliantly with the Bismark brown; all other cells show the characteristic hæmatein stain. The contrast between the two colors makes this the best of several processes we have tried for this purpose.

Biological Laboratory, University of Missouri.

CHARLES THOM.

## A Test of Focal Depth.

In connection with some experimental work in the physical laboratory it was suggested to me to make some measurements of focal depth. If an object on the stage of a microscope is brought into focus, there is for every combination of eye-piece and objective a certain range through which the tube may be elevated or depressed, and during which the clearness of focus seems to undergo no change. To determine the upper and lower limits of this position affords an admirable experiment for practice in accurate settings, and I have collected some of my results into tabular form, thinking that they might be of interest to readers of the JOURNAL.

The microscope used was a Bausch & Lomb instrument of the old type. The pitch of the slow-motion screw was 1-80 inch, and the head was divided into twenty-five divisions, and these divisions, when reading, into fifths; so that motion through one division represented 1-2000 inch, and through one subdivision 1-10000 inch. The specimen chosen for observation was a section of an ivy leaf. Eye-pieces A, B, and C were in turn used, with objectives of one, one-half, one-third, one-fourth, one-sixth, one-tenth, and one-twelfth inch. The last two did not show sufficient depth to yield a series of uniform readings.

In the tables which follow are noted the combination of eye-piece and objective used—ten results in each case, expressed in terms of scale divisions—and finally the focal depth resulting from the mean of these readings.

ONE-INCH OBJECTIVE.

A	B	C
45	26	20
42	26	20
39	27	19
42	28	18
45	27	18
40	27	16
46	26	18
38	28	19
44	28	16
42	29	18
42.3	27.2	18.2

ONE-HALF INCH OBJECTIVE.

A	B	C
8.0	6.0	5.2
8.0	5.6	4.6
7.6	5.2	4.2
8.0	5.0	4.8
7.2	6.2	4.6
6.0	6.0	4.0
6.6	6.6	4.2
7.0	6.2	5.0
6.8	7.0	5.0
7.4	6.6	5.0
7.6	6.4	4.6

## ONE-THIRD INCH OBJECTIVE.

A	B	C
6.2	3.6	2.2
5.6	3.4	2.2
5.6	3.6	2.0
5.0	3.0	2.0
5.2	3.2	2.2
7.6	3.4	2.6
6.0	3.6	3.0
6.2	3.6	3.0
6.4	3.0	3.2
6.0	2.6	3.6
5.6	3.3	2.5

## ONE-SIXTH INCH OBJECTIVE.

A	B	C
2.2	1.2	1.2
2.0	1.4	1.4
2.0	1.8	1.4
2.6	1.2	1.0
1.8	1.4	1.2
2.0	1.2	1.0
2.2	1.0	0.8
2.2	1.2	1.2
2.0	1.0	1.0
2.0	1.2	1.2
2.0	1.2	1.1

## ONE-FOURTH INCH OBJECTIVE.

A	B	C
4.6	3.6	2.2
4.6	3.4	2.2
4.2	3.6	2.0
4.2	3.0	2.0
4.4	3.2	2.2
4.2	3.4	2.6
4.6	3.6	3.0
4.6	3.6	3.0
4.2	3.0	3.2
4.0	2.6	2.6
4.3	2.4	1.7

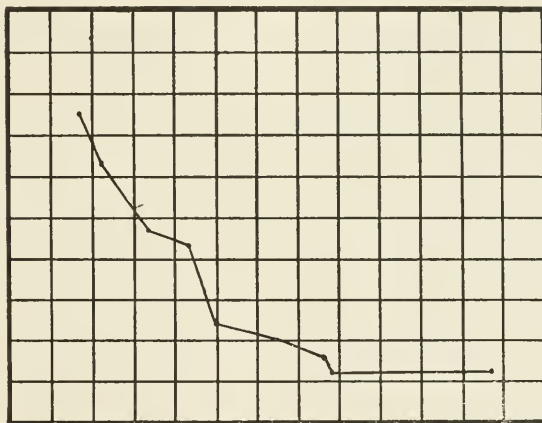
## No. 3 LEITZ.

A	B	C
18	4.0	2.4
20	3.6	2.4
21	4.0	2.2
22	3.8	2.6
19	3.6	2.6
20	4.0	2.0
19	4.2	2.6
20	3.6	2.8
20	3.6	2.6
21	3.2	3.0
20	3.7	2.5

## SUMMARY.

COMBINATION.	FOCAL DEPTH.
1 inch—A.	0.0210 inch.
1 inch—B.	0.0130 inch.
1 inch—C.	0.0090 inch.
1-2 inch—A.	0.0036 inch.
1-2 inch—B.	0.0032 inch.
1-2 inch—C.	0.0023 inch.
1-3 inch—A.	0.0028 inch.
1-3 inch—B.	0.0016 inch.
1-3 inch—C.	0.0012 inch.
1-4 inch—A.	0.0021 inch.
1-4 inch—B.	0.0012 inch.
1-4 inch—C.	0.0008 inch.
1-6 inch—A.	0.0010 inch.
1-6 inch—B.	0.0006 inch.
1-6 inch—C.	0.0005 inch.
3 Leitz—A.	0.0010 inch.
3 Leitz—B.	0.0018 inch.
3 Leitz—C.	0.0025 inch.

The curve which follows is plotted by taking the magnifying powers and the focal depths as coördinates.



Curve showing relation between focal depth and magnification.

The curve approximates an equilateral hyperbola, and indicates that the focal depth varies inversely with the magnifying power.

In order to compare the constancy of the various settings with the magnifying power used, the probable error of the mean was computed for eye-piece A. The results follow:

A—1.	A—1-2.	A—1-3.	A—1-4.	A—1-6.	No. 3 Leitz.
0.57	0.15	0.18	0.05	0.05	0.24

LEON E. RYTHER.

Physical Laboratory, The University of Maine.

## One Way to Number Collections and to Keep Data.

The student just beginning his laboratory course can scarcely realize, at the outset, that material and data are destined to accumulate at a rate that must puzzle him before he is graduated, embarrass him as an instructor, and perplex him when advanced to the head of a department. It seems wholly wrong that students, especially graduate students, who are those sifted out of the great student body to become instructors, should not have the benefit of some regular instruction, or hints at least, touching the matter of keeping notes and cataloguing objects. Unfortunately, we are seldom led in this matter, as in other affairs, to profit by the experiences of others, but are turned out in our several vocations to evolve out of our inner consciousness methods of our own—a course which may foster originality and personality, without necessarily giving the best results.

It is a characteristic of all amateurs and beginners—and not infrequently of some others—to allow the accumulations of several years to go unnumbered and unrecorded, awaiting the time when some system of cataloguing may “turn up.” Too often this means to the end of a natural lifetime, as those can attest who are curators of museums, and who often receive collections of several thousand



unnumbered, unrecorded specimens. This is wholly wrong, and its prevalence is largely due to lack of knowledge. Any method is better than no method; were it otherwise, we might not be emboldened to present our "one way." Cataloguing and recording seems to be a necessary preliminary to subsequent microscopic work in all of the biologic sciences alike, so this "one way" may possibly be entitled to a place in a microscopical journal.

Before the writer had reached senior standing he found himself possessed of several hundred specimens, besides slides, negatives and note-books, etc. This accumulation, much of it of value to the owner, was but prophetic of still greater collections and increasing trouble. A system was straightway decided upon, and however crude or cumbersome, it serves the purpose well, and has been obstinately adhered to, be it good or bad. It is a number which does not repeat, which is not forgotten, which is at once a serial number and a date, and viewed as a whole is an arbitrary number. It is simply the day, month, and year expressed numerically, and boxed in, like a diamond number, so as to preclude the possibility of error in reading it. If an object is found, a memorandum written, an experiment begun, a note-book started, a plate exposed, a book or piece of apparatus added to the shelf, the first thing is to write this date number upon it. Great importance has always been attached to the habitual practice of writing a date upon every transaction, however trifling. It is often the one key to the whole situation. This number proves to be both a date and a catalogue number. The object, whatever it may be, which is obtained, say the first of June, 1899, is numbered 1 

1	6	99
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 the first day, of the sixth month, of 1899,

the next, 2 

1	6	99
---	---	----

the next, 3 

1	6	99
---	---	----

, and so on for each accession of that particular day. If a slide is ground, it may look, when done and catalogued and cross-referenced, somewhat like the cut, Fig. 1. It may not beautify the slide, but it

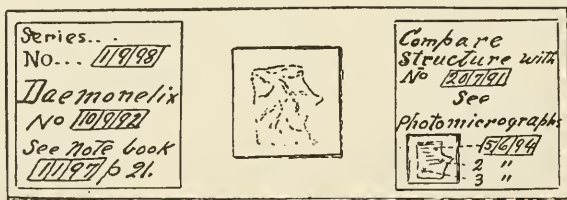


Fig. 1.

will tell where to find facts about it, and that is the important matter.

It is a long number, you say, and so it is. It is infinitely longer than no number, and is longer than 1, 2, 3, at the outset, but it must be remembered that it grows no larger in a lifetime. At the end of a natural lifetime, if one's collection is good for anything at all, it will begin to gravitate naturally toward the repository for such things, the public museum, and there the numbering can be done according to the latest and most approved methods. In the meantime the amateur has had a sort of system, and a certain kind of order has prevailed. It seems to have some advantages in field collecting because the number of each and every specimen is in mind, unless one is particularly absorbed and absent-

minded. The number and the notes are often of more value than the specimen. Certainly the objects alone are ordinarily worthless. If there are several parts to the particular object in hand, one can easily write 1st, 2d, 3d, etc., after the diamond number. To the writer the system seems a convenient and useful one, but we must always remember the personal equation entering into such matters. The very tool which suits one man's hands may not have the "hang" for the next. This date number has a decided advantage in that notes and references are more readily looked up, for one can generally remember the year when certain sections were prepared, photomicrographs taken, specimens obtained, or notes written, and can turn promptly to the year, month, and almost to the day, thus expeditiously looking up the matter or the object sought.

*One Way of Keeping Notes.*—Keeping notes is also a very necessary preliminary to other laboratory work. The student is urged to look with suspicion upon any system of keeping notes which does not admit of indefinite growth and expansion, which does not admit of additions and subtractions, and which cannot be readily arranged with reference to each subject in hand. Members of the

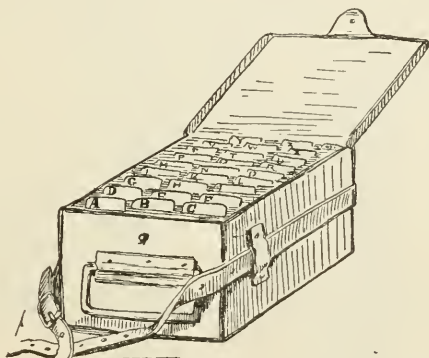


Fig. 2—A sketch showing a portable card catalogue note-book, made of extra heavy tin, japanned, and fitted with light leather sling. Size about  $5\frac{1}{4} \times 5\frac{1}{4} \times 3\frac{1}{4}$  inches.

U. S. Geological Survey, whose note-books have grown into a small library, assert with candor that it would be easier to go into the field and do the work over than to find the notes respecting field work done a few years back. The note-book is too often a jumble of unrelated parts, and when you have many such you wish you had none. Your freight bill would be smaller and your house room larger, and your general perturbation less, at any rate. It is very trying to know that you have notes, yet really haven't them, because they are not available. A remedy is to use punched history paper, or seminar paper with wide margins. Or, still better, especially for short condensed notes, is the card catalogue system. There is no need to describe this system, for every beginner must meet with constant reference to it in this journal, where you must see it figured as well as described. The paper slips or cards of uniform size, and of different weights and colors, are to be had in every city, or can be ordered, together with guide-cards, from any library bureau. The convenience of this system is that one can always have a few slips in his memorandum book, and as soon as a note is

jotted down there is a place for it under A, B, C, etc. And one subsequently hunts for such a note just as he would look up a word in the dictionary.

It is truly a system which is always complete, but never done. In order to have a portable card catalogue for field work, the writer had a small box made of double-strength tin, properly japanned and fitted with leather sling, which proves to be a very great convenience. It is small enough to drop into the corner of one's grip, yet large enough to carry the working notes of a state. When congested, there is an overflow set of drawers. Such notes are always in alphabetical order, and, besides, closely related notes under A, B, C may be grouped together, for which purpose regular folders are furnished by the bureaus. The varied and limitless possibilities of the card catalogue system must be told by the professional librarian; the writer must be content to write as an amateur to amateurs. But this much is certain, anyone can begin, and can soon grow into the system, and can modify it in a great variety of ways to meet his individual wants. For example, clippings may be dropped into envelopes of the same size as the cards, and arranged with them in alphabetical order. Invoice and order blanks are printed upon them in every university, and as a result one's invoice is always written up to date. One's mailing list and exchange list should be kept in the same way, for the little cards act as a tickler to remind one of his debits and credits. At another time the writer hopes to draw upon his tin box for certain notes on the fossiliferous rocks and deposits in the state, and the sectioning and microscopic work which the students are doing in connection with them.

ERWIN H. BARBOUR.

The University of Nebraska.

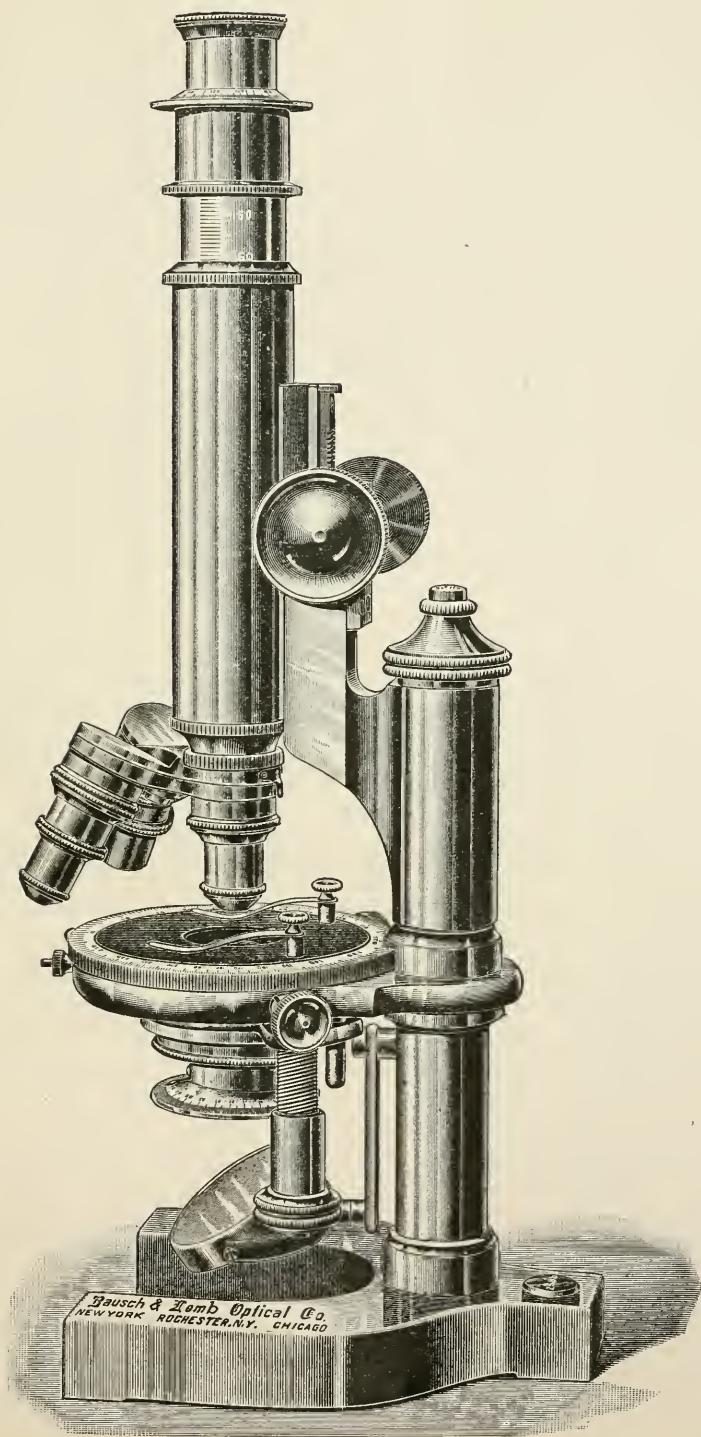
## A Microscope for Micro-chemical Analysis.

It is rather remarkable how slow American chemists have been in realizing the importance of the microscope as an adjunct to every chemical laboratory. This is, perhaps, largely due to the fact that few of our students in chemistry become familiar with the construction and manipulation of this instrument, just as few of them become sufficiently familiar with the spectroscope and its manifold uses; and doubtless also because of the prevailing impression that a microscope is primarily an instrument for the biologist and is of necessity a most expensive luxury. The fact is, however, that this instrument is now far from being a luxury to the chemist, and the time is not far distant when it will be conceded to be as much a necessity in every analytical laboratory as is the balance.

Nor is the apprenticeship to its use in chemical work long nor intricate.

Micro-chemical analysis should appeal to every chemist because of its neatness, wonderful delicacy, in which it is not excelled even by the spectroscope, and the expedition with which an analysis can be made. A complete analysis, intricate though it may be, is a matter of a few minutes rather than of a few hours.

While there is no good reason to believe, as do some enthusiasts, that this new



CHAMOT CHEMICAL MICROSCOPE.



system is to displace the old analysis in the wet way, every chemist should, nevertheless, familiarize himself with the microscope, its accessories, and the elegant and time-saving methods of micro-analysis, thus enabling him to examine qualitatively the most minute amounts of material with a rapidity and accuracy which is truly marvelous; not to speak of the many substances for which no other method of identification is known.

At present the greatest bar to its general use is the absence of any well defined scheme, and the absolute necessity of being well grounded in general chemistry. There are no tables which can be followed in a mechanical way by the student, but on the contrary he is obliged to exercise his knowledge and judgment at every step. For this very reason the introduction of this subject into the list of those now taught is greatly to be desired.

Thoroughly convinced of the desirability of chemical students becoming familiar with the essential features of micro-chemical methods, and of their obtaining some little practice in these methods, the writer deemed it advisable to offer a regular course of work in this subject. Not a course in the manipulation and use of the microscope, but in practical micro-chemical analysis, with special reference to the examination of minute amounts of material and the application of this system to the identification of substances which can be detected in no other way.

At the very outset a most serious obstacle was encountered, the absence of any convenient microscope. There is no doubt that regulation petrographical stands would have answered admirably, but the expense clearly placed them out of the question. These stands were also needlessly complicated for the work to be done. That which was wanted was a compound microscope of as simple construction as possible and fitted with only such accessories as were essential to the work in hand. It was also necessary that the instruments be inexpensive.

The foreign instruments now supplied for this class of work did not seem wholly satisfactory. Negotiations were therefore begun with the Bausch & Lomb Optical Co., regarding the construction of a special stand at a low price, which would fulfill certain specifications, the details of construction being left to them. Thanks to the courtesy and kindly interest of this firm, a neat, efficient instrument has been placed on the market. The writer wishes particularly to acknowledge the excellent manner in which they worked out his suggestions.

At present as in use in our laboratory, where it is giving much satisfaction, the instrument is arranged as follows: (See fig.)

The stand, as will be seen by the accompanying cut, is of the continental type. Since in all the work for which it is intended, the stand is always used in an upright position, it was not thought necessary to provide it with a jointed pillar. The coarse adjustment is by rack and pinion, the fine by micrometer screw. The stage is circular, rotating, provided with centering screws; its circumference is graduated into degrees for measuring crystal angles.

The stage is faced with hard rubber. The substage is adjustable by means of a quick-acting screw. Into the substage is fitted the polarizing apparatus consisting of a Nicol prism of large size and so mounted that by means of a pin fitting into a slot in the substage ring, the prism can always be replaced in

exactly the same position. The polarizer can be swung aside when polarized light is not desired. The prism is so arranged that it can be rotated, and is provided with a circle graduated in degrees. The analyzing Nicol is also provided with a graduated circle, and is so mounted that it fits over and above any eyepiece. The draw-tube of the microscope is furnished with a small projecting pin which fits into a slot cut in the bottom of the tube mounting of the analyzer. This slot lies in the same vertical plane as the zero points of the analyzer, the polarizer, and the stage. The zero points of polarizer and of analyzer are arranged as usual, i. e., for the position of crossed Nicols. It follows, therefore, that when the polarizer is at zero, and is swung in position below the stage, and the analyzer is also at zero and is in position with its slot on the pin, the Nicols are crossed without further adjustment. It is therefore possible to quickly change eye-pieces, drop the analyzer in place, and not be obliged to spend time in adjusting it as is usually the case. The writer is not familiar with any other instrument of corresponding price, in which the polarizing apparatus is so conveniently arranged.

Although the best results are to be obtained, as a general rule, by using high objectives and low eye-pieces, it is quite evident that in micro-chemical work the reverse is true; the rule here being always a low objective and a high eye-piece, thus removing the objective as far as possible from corrosive vapors rising from liquids on the stage. Since, then, an increased magnification is brought about by changing eye-pieces, rather than objectives, the necessity of having the analyzer mounted in some such way as described above becomes apparent.

Each eye-piece is provided with cross hairs at right angles, and a projecting stud fitting into a nick in the upper edge of the draw-tube for lining them.

It will be seen from the above description that the instrument is in reality a simplified petrographical microscope, but it should be borne in mind that it is not intended as a substitute for such a stand, nor is it intended for crystallographic investigations.

In the laboratory of the writer each instrument is provided with one-inch, one-half-inch, and one-fourth-inch objectives; and two-inch, one-and-one-half-inch, one-inch, and one-half-inch eye-pieces. This gives a range of magnification lying between about 20 and 500 diameters, which is amply sufficient for all ordinary analytical work.

The draw-tube of the microscope is graduated as usual; the mirrors, plane and concave, are on a swinging bar.

These instruments have proved so satisfactory that they can be recommended to any one who is in search of a low-priced microscope for chemical work or for food examination.

E. M. CHAMOT.

Chemical Laboratory, Cornell University.

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Dr. Ellet O. Sisson, formerly director of the Microscopical Laboratories in the College of Physicians and Surgeons of Keokuk, Iowa, has been elected director of the Histological Laboratory in the recently consolidated medical schools of that city.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

## VII.

In the preceding chapters the principles and methods of technique have been described in a general way. It is often difficult, especially for a beginner, to apply general principles to specific cases, and, besides, the types which he might select for the preparations might not form a symmetrical collection. Consequently, a series of forms has been selected which will not merely serve for practice in microscopical technique, but will also furnish the student with preparations for a fairly satisfactory study of plant structures from the algæ up to the angiosperms. It is not at all our purpose to discuss general morphology, but rather to answer, by means of sketches and specific directions, the multitudinous questions which confront the instructor in the laboratory. For those who have had a thorough training in general morphology, the following suggestions will be in some degree superfluous. Those who are beginning the study of minute plant structure are referred to the standard text-books for descriptions of the plants mentioned here.

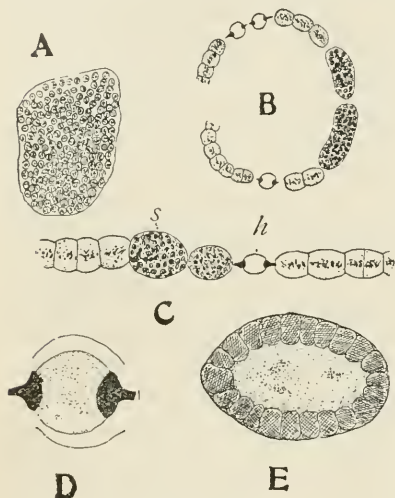


Fig. 7. Wasser Blüthe.

A. *Cœlosphærium* Kuetzingianum. B. *Anabæna* flos-aquæ. C. *Anabæna* gigantea. D and E, a heterocyst and spore of *A. gigantea* drawn from paraffin sections stained in cyanin and erythrosin.

## ALGAE.

## CYANOPHYCEAE.

1. *Wasser Blüthe*.—These forms occur as scums, often iridescent on the surface of stagnant or quiet water. Some of the commonest forms are *Cœlosphærium* and *Anabæna*. Some of the *Chlorophyceæ* also occur as *Wasser Blüthe*. Where the material is very abundant, it may be collected by simply skimming it off with a wide-mouthed bottle, but where it is rather scarce it is better to filter the water through a cloth, and finally rinse the algæ off into a bottle. Enough formalin may now be added to the water in the bottle to make a two per cent. solution. The material may be kept here indefinitely, but after a few hours it is ready for use. If the forms are small, like *Anabæna*, smear a slide lightly with Mayer's albumen fixative, as if for paraffin sections, add a drop of the material and allow it to dry, heat the slide gently

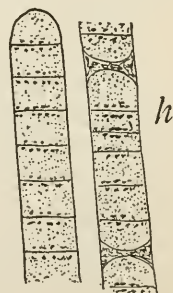


Fig. 8. *Oscillaria*.  
Portions of two filaments, the one at the right showing a hormogonium, h.

to coagulate the albumen or immerse the slide in strong alcohol for a few minutes, and then proceed with the staining. Cyanin and erythrosin is a good combination for differentiating the granules. Delafield's hæmatoxylin, used alone, stains some granules purple and others red. Iron alun hæmatoxylin is excellent for heterocysts. If the forms are large enough to collapse with such treatment, the glycerine method may be employed.

If it is desirable to make paraffin sections, put the material, drop by drop, on a piece of blotting paper until an appreciable layer of sediment is obtained. Get the paper with its material into paraffin by the usual method, taking great care not to wash the algæ off. After imbedding, trim away the paper and dip the block in melted paraffin. Sections can now be cut and stained in the usual manner.

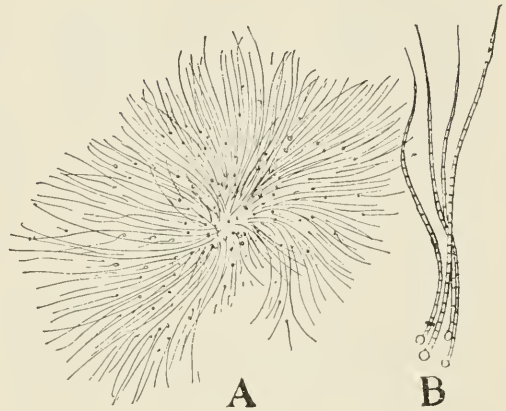


Fig. 9. Rivularia.

A. Nodule crushed under cover-glass. B. Four filaments more highly magnified, showing heterocysts at the base.

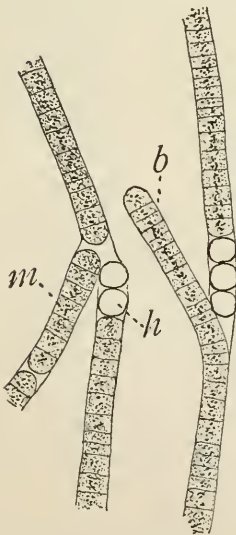


Fig. 10. Tolypothrix.

b. A false branch.  
h. Heterocysts.

*Oscillaria*.—For most purposes it is best to study *Oscillaria* in the living condition. It is readily found in watering-troughs, in stagnant water, on damp earth, and in other habitats. The commonest forms have a deep blue-green or brownish color. For the purposes of identification and herbarium specimens, the material may simply be placed on a slip of mica and allowed to dry. When wanted for use, add a drop of water and a cover, and the mount is ready for examination. For sections or for glycerine mounts fix in chromo-acetic acid.

*Rivularia*.—This form is readily found on the under side of the leaves of water-lilies (*Nuphar*, *Nymphaea*, etc.), but is also abundant on submerged leaves and stems of other plants. It occurs in the form of translucent, gelatinous nodules of various sizes. Chromo-acetic acid gives beautiful preparations, but good results can also be secured from formalin or picric acid material.

The most instructive preparations for morphological study can be obtained by the glycerine method. Stain in eosin or Mayer's hæmalum. When ready for mounting, crush a small nodule by pressing on the cover-glass. Fig. 9 is drawn from such a preparation. The paraffin method is easily applied, since the gelatinous matrix keeps the plants in place. *Glæotrichia*, *Nostoc*, and forms of similar habit may be prepared in the same way.



*Tolypothrix*.—This form occurs as small tufts, either floating in stagnant water or attached to plants and stones.

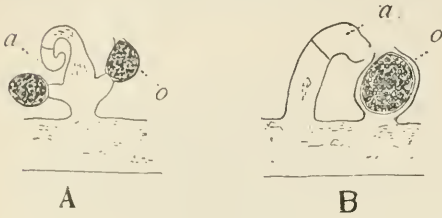


Fig. 11. *Vaucheria*.

A. *Vaucheria geminata*. B. *V. sessilis*.  
a. Antheridia. b. Oögonia.

It furnishes an excellent example of false branching. (Fig. 10.) Scytonema is a similar form which is fairly common. The glycerine method should be employed for permanent preparations, but this, like all small filamentous algæ, may be dried on mica for herbarium purposes.

### CHLOROPHYCEAE.

The ponds, ditches, and rivers of any locality will yield an abundance and variety both of the unicellular and multicellular members of this group. The unicellular and filamentous members, together with such forms as *Volvox*, are best prepared by the glycerine method. The structure is so much more complicated than in the *Cyanophyceæ* that it demands far more care and skill to make good preparations. Chromo-acetic acid is a good killing and fixing agent for the whole group, but Flemming's fluid (weaker solution) seems to be a little better in some instances. Very good results have been obtained by adding about 5 cc. of one per cent. osmic acid to 100 cc. of chromo-acetic acid (Schaffner's formula). A formula which gives satisfactory results with *Spirogyra* may cause plasmolysis with *Cladophora*. The given filament should be placed under the microscope in the fixing agent, and if plasmolysis occurs, the chromic should be weakened or the acetic strengthened until the suitable proportions are determined. This is a slow process, but *Cladophora* and *Vaucheria* are almost sure to shrink without it. About

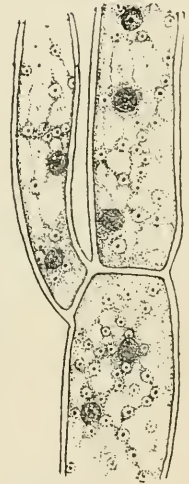


Fig. 12. *Cladophora*. Fixed in chromo-acetic acid, stained in Haidenhain's iron alum hæmatoxylin.

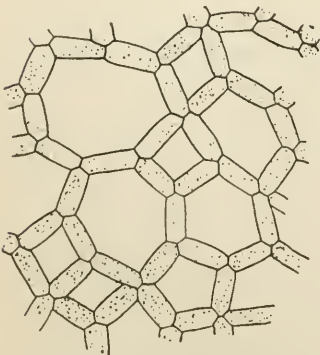


Fig. 13. *Hydrodictyon*.  
A small portion of a young net.

twenty-four hours in any of the chromic series and a four to ten hours washing in water, will be sufficient for members of this group. Only a few of the most familiar forms will be mentioned.

*Vaucheria*.—It is extremely difficult to get mounts showing the nuclei. The following method is sometimes successful:

1. Chromo-acetic acid (Schaffner's formula), 24 hours.
2. Wash in water, 4 to 10 hours.
3. Iron alum, 2 to 4 hours.
4. Water, 15 to 30 minutes.
5. One-half per cent. hæmatoxylin, over night.

6. Wash in water, 5 to 10 minutes.
7. Iron alum until details become clear. This may take only a few minutes, but may take an hour.
8. Wash thoroughly in water, 1 to 4 hours.
9. Ten per cent. glycerine.
10. Mount and seal.

*Cladophora*.—This is found attached to sticks and stones in quiet or running

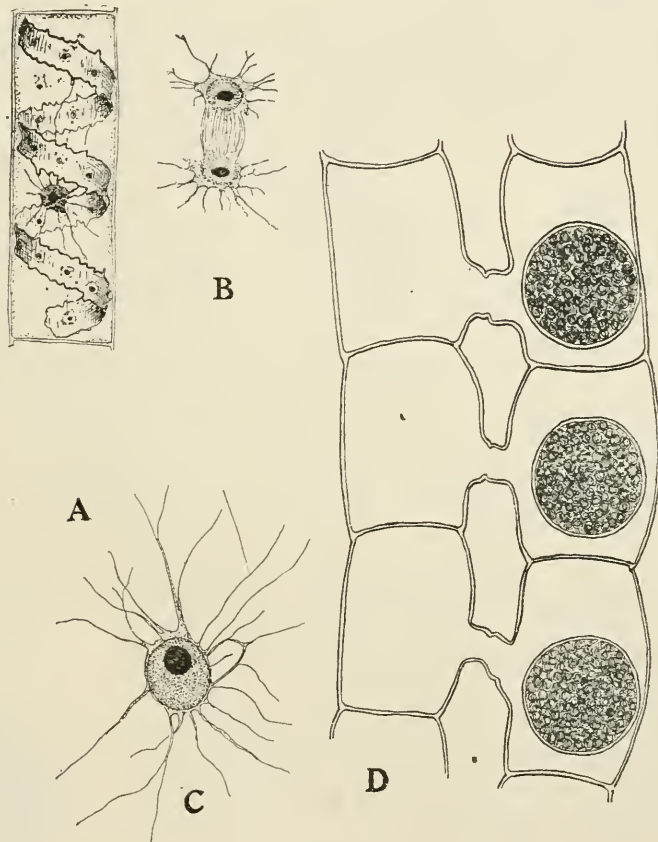


Fig. 14. Spirogyra.

From material fixed in chromo-acetic acid and stained in iron alum hæmatoxylin. A. Single cell showing nucleus, chromatophore, and pyrenoids. B. A nucleus undergoing division.

C. A resting nucleus. D. Zygospores, each showing two nuclei.

water. It is easily recognized by its characteristic branching. (Fig. 12.) The nuclei of the cœnocytic segments are readily brought out by this method. Alum carmine, and Mayer's hæmalum are also good stains for the nuclei.

*Hydrodictyon*.—This is popularly known as the "water-net." Nets of all sizes should be selected for study. The segments are cœnocytic and the nuclei are hard to differentiate except in the younger segments. The method given for *Cladophora* yields good results. The habit is beautifully shown in preparations

stained with eosin. The eosin (one per cent. aqueous solution) should act for about twenty-four hours. Then transfer to one per cent. acetic acid for a few minutes.

If the stain comes out rapidly in the acid, one minute may be sufficient, but if the stain does not wash out, it is better to let the acid act for four or five minutes. Wash thoroughly in water to remove all trace of acid, or the preparation will fade. Transfer to ten per cent. glycerine and proceed as usual.

*Spirogyra*.—This is probably the most widely known of all the algæ, and, fortunately, it is rather easy to obtain beautiful and instructive preparations. The following is a good fixing agent for most *Spirogyras* :

Chromic acid,  $\frac{4}{10}$  gram.

Glacial acetic acid,  $\frac{6}{10}$  gram.

Water, 99 cc.

The addition of one cc. of osmic acid seems to improve it without causing any blackening. Flemming's weaker solution is excellent. If it causes too much blackening, as it probably will, the material, after being washed, should be placed in weak peroxide of hydrogen (one part  $H_2O_2$  to three parts  $H_2O$ ) until the blackening due to osmic acid disappears. After a moment's washing in water it is then ready for the stain. The iron alum hæmatoxylin, as just described, brings out the nuclei and pyrenoids with great distinct-

ness. A few minutes in aqueous eosin after the last washing in water, often gives a beautiful differentiation, but the preparations will be quite inferior if the eosin is allowed to stain too deeply. Mayer's hæmalum is a better stain for stages in conjugation.

It is difficult to get *Spirogyra* into paraffin without shrinking, but it can be done. Watch carefully and note where plasmolysis occurs. There will probably be little or no trouble until the transfer from 100 per cent. alcohol to the clearing agent. Make this transfer as gradual as may be necessary. After the pure xylol or other clearing agent is reached, add a lump of paraffin large enough to saturate the clearing fluid at a temperature of 40 to 45 degrees C. Allow the xylol to evaporate at this temperature and imbed as usual, taking care to keep the filaments as nearly parallel as possible. Many elegant combinations, like cyanin and erythrosin, fuchsin and iodine green, safranin-gentian-violet-orange, and others not available for glycerine preparations, can be used with paraffin sections. It is comparatively easy to get any such alga into celloidin. Safranin and Delafield's hæmatoxylin then makes a good combination.

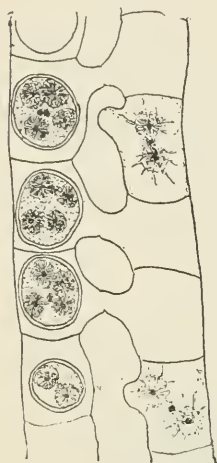


Fig. 15. Zygnema.

The filament at the left shows three zygospores and one parthenogenetic spore which is distinguished by having only two chromatophores. The filament on the right shows two cells, each with a pair of stellate chromatophores. Drawn from material fixed in two per cent. formalin and stained in iron alum hæmatoxylin.

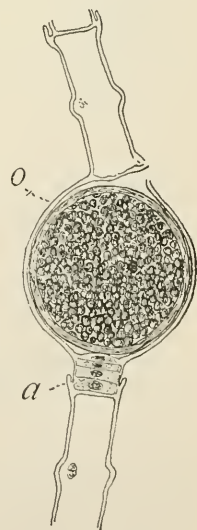


Fig. 16. Oedogonium nodulosum.

a. Antheridia. b. Oogonium. Drawn from material fixed in one per cent. chromic acid, and stained in Mayer's hæmalum.

*Zygnema*.—Use the same methods as for *Spirogyra*. In staining conjugating material the stain should be extracted until the four chromatophores of the zygospore become distinct. The nuclei are comparatively small and unsatisfactory. The stellate chromatophores are well brought out by alum carmine. If iron alum hæmatoxylin and eosin are used, the eosin may well be much deeper than in case of *Spirogyra*.

*Oedogonium*.—In selecting material it will be better for teaching purposes to choose the larger monœcious forms. The nuclei, pyrenoids, and chromatophores are easily differentiated. Mayer's hæmalum is a good stain, especially for the antheridia. Alum carmine or eosin will bring out the "caps."

*Chara*.—This form is so large and coarse that it hardly pays to mount it in glycerine. If a glycerine mount is desired to show the antherida and oögonia in position, spin a ring of cement on the slide, thus making a cell in which small portions of the plant may be mounted. For paraffin sections select the tip of the plant,

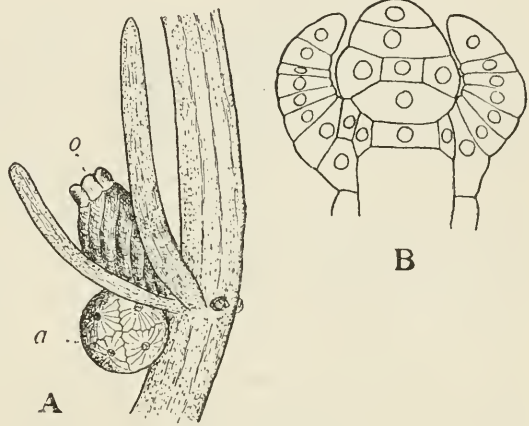


Fig. 17. *Chara*.

A. Portion of a branch showing an antheridium, a, and an oögonium, b. B. Median longitudinal section of an apical cell. Drawn from a preparation fixed in chromo-acetic acid, and stained in Delafield's hæmatoxylin.

a piece not more than a quarter of an inch in length. In the smaller species this may show not only the large apical cell, but also various stages in the development of antheridia and oögonia. Delafield's hæmatoxylin is a very good stain for the apical cell and for the development of antherida and oögonia. The later stages in the development of antherozoids are brought out more clearly by the safranin-gentian-violet-orange, or by cyanin and erythrosin.

Good preparations showing shield, manubrium, capitula, and filaments, may be obtained by staining in bulk in alum carmine and then crushing the antheridium under the cover-glass after the specimen is in balsam.

(To be Continued.)

## Growing Anaerobes in Air.

Anærobic bacteria (at least in the case of tetanus, symptomatic anthrax, and malignant œdema), grow readily in glucose-agar stick cultures, without any precautions to exclude oxygen, if the tubes be placed in the steam sterilizer for ten minutes, and then quickly cooled just before inoculating, although no growth, or only a scanty one, took place in the same medium under identical circumstances if this precaution was omitted; the explanation being that the free oxygen is driven out by the heat employed, and growth takes place before the medium has had time to re-absorb sufficient from the air to interfere with growth.

W. W. ALLEGER.



# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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OUR contributors will be gratified to know that during a recent tour of the Western States and the Pacific Coast we found the suggestions regarding apparatus and equipment for laboratories, and the methods which have been published in actual use in almost every educational institution visited.

The general expression is for "more of the same kind" of papers as those which have been appearing.

The busy teacher or investigator who lays aside his work to prepare a description of a particularly effective

method, or a practical piece of apparatus for the JOURNAL, has the satisfaction of knowing that it will be read and appreciated by many a teacher or individual worker who would otherwise be obliged to use an inferior process for lack of time to work out the problem for himself.

\* \* \*

The development of biological work in higher educational institutions is well indicated by the extensive plans being made in all the newer universities for laboratories, and the great care which is being given to the detail of their equipment in order to secure for the student the best conditions for work, and the most suitable appliances. The wisdom of the University of California's expenditure of \$50,000 for plans for her buildings and grounds is unquestioned. The time when a laboratory can be installed in any sort of building, be conducted without an adequate system of working and suitable appliances, intelligently handled, and still be a success on account of the connection therewith of one or two brilliant investigators, is rapidly passing, if not altogether gone by.

Even the undergraduate is coming to see the difference in the results, and is refusing to patronize the laboratory in which he is obliged to work under unnecessary difficulties.

\* \* \*

Continuing the papers on representative American laboratories, we have, during the summer, visited a number of the leading universities of the West. Descriptions, with illustrations made especially for the purpose, will appear in the JOURNAL during the coming year. These papers are designed to enable those who have the planning and arrangement of new laboratories, or the re-arrangement of old ones, to secure the benefit of what has been done in the more advanced institutions. It is with this end in view that all connected with the various laboratories visited have so willingly aided in the collection of the necessary data.

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One hundred bound reprints are furnished gratis to authors whose papers are published in the JOURNAL, if desired. Reprints should be requested when proof is returned.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Coulter, John M.** Plant Relations. A First Book of Botany. New York, D. Appleton & Co. Pp. VII+264; 206 figs., 1899.

This is quite different from any of the recent text-books which have aimed to meet the needs of the secondary schools. It is dominated by ecology, written from the standpoint of Warming, and also contains some of the fundamentals of physiology, but there is scarcely a trace of morphology. The book is designed to suggest work for half a year, and a companion book devoted to morphology, evolution and classification, also designed to suggest a half year's work; will appear soon. The elegant literary style, the refreshing freedom from technical terms, and the inherent interest of the subject matter, make the book a delightful one to read. The illustrations, as a rule, are excellent and many of them are new. It is a departure to place ecology before morphology, and the value of this arrangement can be determined only by actual experience. The companion book will enable each teacher to adopt what he regards as the most teachable sequence. A pamphlet of twenty pages containing suggestions to teachers accompanies the book.

C. J. C.

**Sargent, Ethel.** On the Presence of Two Vermiform Nuclei in the Fertilized Embryosac of *Lilium Martagon*. Proc. of the Royal Soc. 65: 163-165, 1899.

The brilliant results of Nawaschin and Guignard on the fertilization of *Lilium* are supported by the present brief paper. Miss Sargent finds that during fertilization the male nucleus is applied to the female nucleus, while the second male nucleus is applied to both the polar nuclei. In one case, in which the two polar nuclei were not in contact, the much elongated "antherozoid" united them like a bridge. In several preparations it was noted that the pollen tube, after fertilization had taken place, contained two small nuclei. Since both generative nuclei are accounted for, these are probably due to the division of the tube nucleus.

C. J. C.

**Von Schrenk, Hermann.** A Disease of *Taxodium* known as Peckiness, also a Similar Disease of *Libocedrus Decurrens*. Reprinted in advance from the eleventh annual report of the Missouri Bot. Garden. Pp. 1-55, pl. 1-6, 1899.

It has been estimated that thirty per cent. of the lumber obtained from the bald cypress, *Taxodium distichum*, shows the disease described in this paper. The decay occurs in sharply localized areas, giving the logs in cross section the appearance of having been bored full of holes, varying in diameter from one-fourth to three-fourths inches. This feature has led lumbermen to apply the term "pecky." The wood between the holes is apparently perfectly sound, and shows no peculiarity in microscopic structure. The cell-walls are changed into compounds which diffuse through

the walls and fill the cells surrounding the decayed center. A fungus with strongly marked characters grows within the decayed centers and between them without injuring the intervening wood. The fungus was not found in fruit, and could not be identified. The decay does not continue after the tree has been cut down, and the "pecky" lumber seems to be about as durable as the sound, although its commercial value is considerably less. The disease of *Libocedrus* presents similar characters, and it is probably caused by the same fungus. The article is interesting whether viewed from a scientific or an economic standpoint.

C. J. C.

Schmidle, W. Einiges über die Befruchtung, Keimung und Harrinsertion von Batrachospermum. Bot. Zeit. 57: 125-135, pl. 4, 1899.

This work was undertaken with a view to testing the validity of the results given by Davis\* in his work on the Fertilization of Batrachospermum.

Davis' results are briefly, these: (1) The trichogyne is an individual cell. (2) In fertilization there is no fusion between the nuclei of the spermatium and the trichogyne, or between those of the spermatium and the carpogonium. (3) Fertilization consists only in the fusion of the protoplasm masses of the spermatium and trichogyne. (4) After fertilization the cells of the carpogonium and the spermatium begin to fragment. (5) In old trichogynes there are differentiated parts of the protoplasm of a green color. It is suggested that they function as chromatophores and that the long life of the trichogyne after fertilization is due to them. (6) The antherida have reduced chromatophores.

The present writer confirms the results mentioned under 4, 5, and 6, but believes that the statements given under 1, 2, and 3 are largely erroneous. He does not believe that Davis is right in regarding the trichogyne as an independent cell. The spermatia, when in contact with the trichogyne or near the trichogyne, are provided with a membrane and almost always have two nuclei. One of these nuclei, surrounded by its protoplasm, wanders into the trichogyne, and the other usually follows, the narrowness of the canal apparently offering no hindrance to the passage of the nucleus from the spermatium to the trichogyne. A series of stages shows that there is a union of the sperm-nucleus with the nucleus of the carpogonium, as would be expected from Willes' work on *Nemalion multifidum*, and Ottmann's researches on various members of the Rhodophyceæ.

The plates will hardly bear comparison with Davis' excellent drawings of Batrachospermum, but the evidence against points 1, 2, and 3, as stated above, together with positive evidence that there is a fusion between the nucleus of the spermatium and that of the carpogonium, makes it almost certain that Batrachospermum is not so exceptional in its fertilization as has been thought.

C. J. C.

#### RECENT LITERATURE.

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## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
Agnes M. Claypole, Sage College,  
Ithaca, N. Y.

### CURRENT LITERATURE.

Beard, J. D. Functions of the Thymus. Lancet, p. 1, Jan. 1899.

The author started in the study of this problematical organ several years ago, and has but now reached conclusive results. His subject was *Raia batis* the smooth skate. At the period when leucocytes first appear there are no lymphoid structures of any kind, but the primordia of the thymus are present and from their epithelial cells the first leucocytes are formed. When the embryos are about 28 mm. and upward in length, the thymus sets to work in earnest to form leucocytes, and these wandering cells begin to emerge in crowds causing larger or smaller "breaks" on the contour of the thymus.

It is Kölliker, who showed that leucocytes arise in the thymus from its original epithelial cells. Gulland observed that the first leucocytes formed are found in the mesoblast near the thymus. Now Dr. Beard has shown that the first leucocytes arise in the thymus from its epithelial cells and that thus it is the parent source of the leucocytes of the body.

A. M. C.

Grassi, B., Bignami, A. and Bastianelli, G. Mosquitoes and Malaria. Atti. R. Accad. Lincei (Rend.), 8: 21-8, 1899.

The authors come to the conclusions that the *Hæmosporidia* or *Hæmamœbinæ* pass in man through a chapter of their life cycle characterized by the long duration of the amœboid phase and the absence of the encapsulated stages. It is undetermined how many times they reproduce, but they also give rise to forms which are sterile in man. The last stage reaches the intestine of the adult *Anopheles claviger* Fabr. and develops as typical sporozoa forming an enormous number of sporozoites which accumulate in the salivary glands and return to man when the mosquito punctures the skin. It is claimed to be a demonstrated fact that the parasites pass directly from man to mosquito and from mosquito to man, but other species of Diptera besides *Anopheles claviger* may also share in the dissemination, and the open question of passage from the parent insect to its offspring is left undiscussed.

A. M. C.



**Jenner, L.** New Preparation for Rapidly Fixing and Staining Blood. *The Lancet*, 688-689, 1 pl. 1899.

The author uses a mixture of equal parts of 1.2—1.25 per cent. of Grüber's water-soluble eosin in distilled water, and a one per cent. solution of Grüber's medicinal methylen blue. These are mixed in an open basin, stirred well with a glass rod, filtered after 24 hours, and the residue dried in the air or an incubator at a temperature not exceeding 55 degrees C. When dry this is powdered, shaken with distilled water, washed on a filter, dried and again powdered.

For use .5 gram is dissolved in 100 cc. of pure methyl alcohol and the filtrate used as the fixative stain. Cover glass preparations are made by pouring a few drops of the solution on a dry blood film, allowing it to remain for 1 to 3 minutes. The stain is poured off, the cover-glass rinsed in distilled water for 5 to 10 seconds, until the film becomes pink. The cover-glass is then dried in the air. Red corpuscles are terra-cotta colored, the nuclei of white cells blue, platelets mauve, the granules of the polymorphonuclear white cells and myelocytes are red; those of the basophile or mast cells dark violet, and bacteria filariæ and malarial parasites blue.

A. M. C.

**Joukowsky, D.** Reproduction of the Ciliata. *Zoöl. Centralbl.* 6: 42-43, 1899.

The author has repeated Maupas' famous experiments, and the somewhat varying results reached by the two ob-

servers show us the need for caution in considering this question.

In *Pleurotricha lanceolata* over 458 generations were observed without the occurrence of degeneration. The size of the individual depends on nutrition mainly; the rate of multiplication varies markedly with the temperature; disturbances are apparently produced by the accumulation of waste products in the water, and degeneration seems to be due not merely to the number of the generations, but also the rapidity of their succession.

*Paramœcium caudatum* showed no nuclear degeneration even after 5 month's culture, but the cilia were markedly reduced and the animals were sluggish in consequence.

In *P. putrinum* effective conjugation between the descendants of one individual was observed, but the author admits that this probably has its limits.

A. M. C.

**Negri, A.** Ueber die Persistenz des Kernes in den roten Blutkörperchen erwachsener Säugthiere. *Anat. Anzeig.* 16: 33-38, 9 figs. 1899.

The author has taken up some observations made by Petrone (1897-98) in several different articles on the existence of a nucleus in the adult mammalian red

blood corpuscle. Negri repeated Petrone's experiment using his methods which give exceptionally definite and clear results. The best preparations were obtained by treating the blood with one-tenth per cent. osmic or picric acid and staining in formic acid carmine. The blood used was chiefly that of man and the dog, and in every point Petrone's rather startling observations were corroborated. Both investigators found that in the adult corpuscle there is present a small, sometimes central, sometimes eccentric body showing a differentiation into a centrally darker and peripherally lighter area. Negri also found these structures in adult corpuscles of man and dog and laid particular emphasis on tracing the

change from the nucleated corpuscle of the embryo to the blood cell of the adult. All the steps of the process have not been observed, but the author believes that a completion of his studies will show that the central or excentric body of Petrone, found also by himself, is the remains of the nucleus of the embryonic blood cells.

A. M. C.

**Peabody, James Edward, A. M.** Laboratory Exercises in Anatomy and Physiology. 79 pp. Henry Holt & Co., 1898.

In this little book Mr. Peabody has attempted a thorough and logical application of the laboratory method to the study of human physiology in the secondary schools, and has succeeded admirably. As he remarks in the preface, "The following laboratory directions aim primarily to familiarize the pupil with the working of his own organs of motion, circulation, respiration, and digestion."

The book opens with a chapter on "The Skeleton and Muscles." The nature of the material used in much of the work is well illustrated in the first section on the "Structure of Bones," where the material required is as follows: "A clean rib of lamb or pork cut smoothly across the end; two clean soup-bones, one sectioned transversely, the other divided into halves lengthwise."

In the section "Study of the Muscles" the pupil identifies, as far as possible, the muscles of the arm and hand and their method of action. Then the structure of muscle is studied from lean meat, then the structure and function of joints.

The second chapter deals with "Foods," and here are given, in such manner as to render their value clear to the student, the simpler tests for starch, sugar, nitrogenous substances, etc. Following this is a chapter on "The Processes of Digestion and Absorption," in which experiments upon the digestibility of the various nutrients are carried out and the principles of osmosis are illustrated. Chapter IV deals with "The Blood and Circulation," and includes work upon blood, a study of the mammalian heart, observation of the circulation in the tail of the tadpole, and observations upon the student's own pulse.

The other chapters of the book are as follows: "Oxidation and its Products," "Respiration," "The Skin," "Excretion," "Nervous System," "Supplementary Work." In conclusion are given "Rules for the Use of the Compound Microscope," and "List of Apparatus and Chemicals for a Class of Twenty-four."

Throughout the book the admirable plan is followed of limiting the material for study as far as possible to common objects or substances which the student knows as foods or has met with in some other way.

Many interesting and valuable exercises are necessarily debarred from the book in consequence of the fact that, as the author remarks, "physiology unfortunately precedes physics and chemistry in the ordinary high-school courses of study." Thus, the author has regarded it as necessary to omit any thorough consideration of levers, and the senses of sight and hearing are left entirely out of consideration, because of the necessity of some knowledge of physics for any adequate understanding of them. Indeed, in this respect the book illustrates clearly the disadvantages connected with this position of physiology in the high-school curriculum. Physiology, even in its simplest form, must be almost wholly an application of the principles of physics and chemistry,

and it would seem that the system which teaches so complex a subject as physiology before physics and chemistry is capable of improvement. As the human race passed beyond the naive stage and began to seek for explanation of the world, it was the external, inorganic world which first attracted attention—the falling stone, the rain, the dew, ice, fire and its effects, and other simple physical and chemical phenomena. Not until a much later stage did it become evident that the principles underlying physics and chemistry are illustrated in the living body. It would seem that the logical method of teaching is, if we may so speak, the evolutionary one.

In this book the difficulties under which the author labors in endeavoring to make clear the nature of various physical and chemical phenomena are evident, and the scope of the work is thus very limited. The attempt, however, is most praiseworthy, and the defects of this kind which appear should not be charged to the author.

The chapter entitled "Supplementary Work," and especially the section on the study of bacteria, is exceedingly valuable, affording the student, as it does, a realization of the fact that bacteria are present in air, water, dust, milk, etc., and illustrating some simple methods of sterilization and disinfection.

The directions for observations and experiments which are to be carried out by the student at home, and reported upon, constitute what appears to be a very valuable feature of the book. Exercises of this kind are distinguished from class demonstrations and exercises for the pupil in the laboratory. By this independent work it must be possible in many cases to secure a more active interest on the part of the student, and of course the advantage in the increased amount of work possible is obvious.

The style is in general concise and clear. Directions are brief and yet sufficient. Each experiment or observation is numbered, and directions for preparing tables for reporting results are given wherever necessary. The type is clear and of convenient size, and the book is remarkably free from typographical errors. It is bound with alternate blank leaves for notes, which is always a useful provision in a laboratory manual.

Altogether, the plan of the book and the idea upon which it is based are most praiseworthy. It should undoubtedly prove a very useful addition to the list of laboratory manuals for the study of biological science in the secondary schools.

C. M. CHILD.

#### RECENT LITERATURE.

**Herrera, A. S.** Recherches sur le protoplasme artificiel. Bull. de la Soc. Zool. de France, **24**: 20, 1899.

**Maximow, A.** Ueber die Structur und Entkernung der rothen Blutkörperchen der Säugethiere und über die Herkunft der Blutplättchen. Arch. f. Anat. u. Phys. **33-82**, 1 pl., 1899.

**Allis, E. P.** On Certain Homologies of the Squamosal, Intercalars, Exoccipital and Extrascapular Bones of *Amia Calva*. Anat. Anz. **16**: 49-72, 1899.

**Herxheimer, K.** Ueber die Structur des Protoplasmas, der menschlichen Epidermiszelle. Arch. f. Mikr. Anat. **53**: 510-546, 1 pl., 1899.

**Schönichen, W.** Der Darmkanal der Onisciden und Aselliden. Zeits. f. Wiss. Zool. **64**: 145-178, 2 figs, 1 pl., 1898.

**Silvestri, F.** Ricerche sulla fecondazione di un animale a spermatozoi immobili. Ricerche fatta nel Lab. di Anat. nom della R. Univ. di Roma, **6**: 255-265, 2 pls. 1898.

**Pfannenstiel, J.** Noch ein Wort zur Discussion ueber die Syncytiumfrage. *Centralbl. f. Gynäkel.* No. 48, 1314-1319, 1898.

**Bauer.** Ueber das Verhältniss von Eiweis zu Dotter und Schaale in den Vogeleiern. *Biol. Centralbl.* 19: 320, 1899.

**Thilo, O.** Die Entstehung der Luftsäcke bei den Kugelfischen. *Anat. Anz.* 16: 73-87, 1 pl., 1899.

**Needham, J. G.** The Digestive Epithelium of Dragonfly Nymphs. *Zool. Bull.* 1: 103-113, 10 figs., 1899.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review to H. H. Waite, 709 North University avenue, Ann Arbor, Michigan.

**Marzinowsky, E. J.** Ueber eine neue Methode der Differentialfärbung der Microorganismen der menschlichen und Vögeltuberculose, Lepra und Smegma. *Centrlbl. f. Bakt.* 25: 762-764, 1899.

For staining, a water solution of carbolic fuchsin was used (2 parts water, 1 part carbolic fuchsin) and Löffler's methylen blue. The preparations were stained in the water solution of car-

bolic fuchsin for from 3 to 5 minutes. (The author does not state as to whether heat was used.) After carefully washing in water the preparations were next stained for 2 to 3 minutes with Löffler's methylen blue. After these manipulations all the preparations were stained an intense blue. This intense and permanent staining is probably due to the fact that the water solution of carbolic fuchsin acts as a mordant for the subsequent staining with methylen blue.

The *Bacillus tuberculosis hominis* could not, in spite of the intense staining of the sputum streaks, be stained by this method. Moreover, the tubercle bacilli in sections from tuberculous organs were not stained by this method. The *Bacillus tuberculosis avium* is stained tolerably easily in this way. The sections were stained with the water solution of carbolic fuchsin from 6 to 8 minutes, and after careful washing in water were placed in Löffler's methylen blue for 5 minutes. The sections were further treated in the ordinary way—alcohol, oil of bergamot, xylol, balsam. The tubercle bacilli (aviary) were stained red, all other bacteria and the nuclei of the cells of the tissue were stained blue; protracted exposure of the sections to alcohol did not lead to their decolorization. If the sections were left still longer in the methylen blue, the bacteria were stained a lighter tint and were changed from a red to a light rose.

The *Bacillus lepræ* was very easily stained by this method (2 to 3 minutes in the water solution of carbolic fuchsin and  $1\frac{1}{2}$  to 2 minutes in methylen blue). The rods appear red and granular. Alcohol decolorizes them tolerably quickly and they were decolorized still more readily with alcohol after exposure to methylen blue for a longer period (10 minutes).

The *Bacillus smegmæ* also stains red. The preparations were stained in the water solution of carbolic fuchsin for from 4 to 5 minutes, with Löffler's methylen blue 2 to 3 minutes.

If the preparations were left in methylen blue for a longer time (10 to 15 minutes) the smegma bacilli took on a violet color, and on longer treatment grad-



ually became blue. By this method the *Bacillus tuberculosis hominis* is sharply differentiated from the other three—*Bacillus tuberculosis avium*, *Bacillus lepræ*, and *Bacillus smegmæ*—since it is not stained, while all the others are easily stained. One might easily confound the *Bacillus tuberculosis avium* with *Bacillus lepræ* and *Bacillus smegmæ*, since they all take the same stain (red). However, there is shown a difference even here. If the *Bacillus tuberculosis avium* is treated for 10 minutes with Löffler's methylen blue it does not lose its red color, whereas the *Bacillus lepræ*, treated in the same way, is completely decolorized and the *Bacillus smegmæ* loses its red color and becomes violet or blue.

The usefulness of the above mentioned method is shown in the examination of urine to distinguish the *Bacillus tuberculosis* from the *Bacillus smegmæ*, as well as in the examination of tuberculous or leprous affections of the internal organs, to differentiate between the *Bacillus lepræ* and the *Bacillus tuberculosis*.

This method, as has been seen, is useful also to distinguish the *Bacillus tuberculosis hominis* from the *Bacillus tuberculosis avium*. H. H. W.

**Plimmer, H. G.** Vorläufige Notiz über gewisse vom Krebs isolierte Organismen und deren pathogene Wirkung in Tieren. Centrbl. f. Bakt. 25: 805-809, 1899.

This work covers the examination of 1278 cases of cancer from various organs and parts of the body. In only nine of these cases were there present

in the cell, bodies which the author regarded as parasites. In some of the cells as many as 36 of these bodies were found. Believing that these organisms stood in some causal relationship to the disease, cultures were made from nine of the cases, and in one of the two which contained enormous numbers of these organisms an organism was isolated which was pathogenic for certain animals and maintained its virulence for several months.

The cancer from which the organism was isolated was from the breast of a 35-year-old woman. This cancer was of two-months standing and was in rapid proliferation at the time of the operation. Immediately after the operation a section of the cancer was examined and an enormous number of bodies were found in the cells. Thin sections cut from the cancer with a sterile knife, and some of the fluid scraped from the cut surface were placed in a flask which contained a medium made up of a broth prepared from the cancer itself, and to this was added, after careful neutralization, 2 per cent. of glucose and 1 per cent. of tartaric acid.

The cultures were grown under anaërobic conditions in an atmosphere of hydrogen. Such cultures maintain their virulence and are as effective after four months as when recently isolated.

The organism apparently belongs to the *saccharomyces*. Microscopically they are round, often growing in clumps, with a darkly staining center and in most cases with a highly refractive, thin capsule which at times shows a double border. In many of the younger cells no capsule is seen. They vary in size from 0 to 0.04 mm. to 0 to 0.4 mm. They multiply by budding, and the author believes he has also observed endogenous spores. Grown in the above mentioned medium, after forty-eight hours there is a cloudiness which increases for six days, and then the medium becomes clear without the formation of a scum.

On this medium, small round colonies develop which are at first white, but after a few weeks become yellow. Gelatin is not liquefied and development is never great.

On potatoes a thick layer is formed which at the end of two weeks spreads over the surface of the potato and becomes yellowish-brown in color. Grown aërobically it soon loses its virulence.

In only one experiment was an epithelial surface inoculated (the cornea). In this case there was a marked proliferation of the epithelium with an appearance of the organism in the cells and excessive irritation.

*Conclusions.*—There are certain cancers, of very rare occurrence, which contain cells filled with many bodies described by Ruffer and others as parasitic protozoa. From their rare occurrence and their exceedingly acute course they are thought to have a different origin than the ordinary cancer, but there is no greater difference between them than between the acute and the chronic tubercle. By suitable means the contents of these cells can be isolated and grown outside the body. These cultures inoculated into a susceptible animal are capable of producing death with the formation of tumors, of endothelial origin with one exception. From these proliferations pure cultures can be obtained, which, when inoculated into susceptible animals, produce similar tumors. H. H. W.

**Novy, Frederick G., Sc. D., M. D.,** University of Michigan. *Laboratory Work in Physiological Chemistry.* Second edition, revised and enlarged, with frontispiece and twenty-four illustrations, pp. 326, 1898. Ann Arbor: George Wahr.

"Every medical student should receive thorough drill in the laboratory, not merely in so-called urine analysis, but in the broader field of physiological chemistry. He should be taught to

observe and to reason; to correlate the facts brought out in the laboratory in their relation to physiology, hygiene, and disease."

The above, taken from the preface of this work, strikes the key-note of laboratory work for medical students. It is to be regretted that all medical schools do not require a knowledge of elementary chemistry for entrance. If this were the case, the student would be better prepared for other work, and the time now devoted to elementary chemistry could be given to a more extended study of physiological and pathological chemistry.

This volume starts with a chapter devoted to the study of "Fats," giving brief explanatory descriptions of the source, the character, and composition of fats; the action of the various ferments upon them, and the various theories of absorption. With this introduction, the student is given directions for the performance of nineteen experiments on fats and their decomposition products, which must needs fix, and impart to the student, an amount of knowledge which will prove of inestimable value.

The second chapter is devoted to the "Carbohydrates," the Tollens classification being used. The preliminary descriptions contain a large amount of information in a comparatively small space. The subjects covered in this chapter are pentoses, hexoses, glucose, cane sugar, lactose, maltose, starch, dextrin, glycogen, and cellulose, together with a brief description of the polariscope, and its use as applied to the examination of sugars. The experiments

are carefully selected, and the directions for their execution clearly and concisely given.

The next chapter is given up to a consideration of the complex group of nitrogen-containing bodies, variously designated by different authors, but which the author calls "Proteins." After giving the classification of the proteins, essentially that of Wroblewski, the individuals of the classes are considered, together with the various reactions by which they are separated and identified.

We would take exception to the following statement: "In icteric conditions, the saliva does not contain bile constituents." The investigations of Fenwick prove the presence of bile pigments in the saliva in certain diseases.

The section on "Gastric Juice" is quite exhaustive and valuable. We believe, however, that it would have been improved if a few experiments on the action of formic aldehyde on proteins, and the difficult digestibility of the formaldehyde-proteids had been added. This would be of especial value, as the use of formaldehyde as a preservative for milk seems to be on the increase.

Again, in the chapter devoted to milk, the experiments are well selected, but the addition of a few qualitative tests for the presence of some of the common preservative agents would have increased its value very much.

Chapter XI is devoted to quantitative analysis, and gives the student a very clear and concise, though brief, outline of so much quantitative analysis as may be necessary for medical students.

The volume closes with an excellent table for the examination of urine, and some good plates of urinary sediments.

On the whole, the work is clear and concise, and still sufficiently ample to impart an excellent working knowledge to the medical student. We can most heartily recommend it to the careful consideration of teachers of chemistry in the medical schools of the country.

JOHN A. MILLER, PH. D.

Berlin.

**Hueppe, Dr. Ferdinand.** *The Principles of Bacteriology.* Translated by E. O. Jordan, University of Chicago, pp. 455. The Open Court Publishing Co.

Professor Jordan is to be thanked by American bacteriologists for putting into clear English this work of Dr.

Hueppe. The work is more than a simple outline of the principles of bacteriology, since it contains an exposition of certain aspects of this subject that do not commonly receive treatment. Dr. Hueppe's views upon certain phases of bacteriological problems are in some respects peculiar. In this work he places himself in opposition to the school of Koch in regard to the relation of bacteria to the problem of the cause of disease.

In the first three chapters he gives a general account of bacteria, the vital phenomena connected with their life, and a description of the most important pathogenic bacteria. The chapter upon the vital properties of these organisms is especially important, containing valuable information concerning the chemistry of bacteriological foods and poisons.

It is in the following chapters upon the cause of infectious disease, and kindred subjects, that the suggestive part of the work begins. In brief, Dr. Hueppe denies the idea that "specific" bacteria are the cause of specific

diseases, thus taking a position quite at variance with that of most writers on bacteriology. Of course Dr. Hueppe is perfectly familiar with the great mass of facts upon which the conclusions of the Koch school are based. He is fully aware that diseases are produced in animals by inoculating them with definite bacteria, and he does not question that the bacteria are the stimulating influence which originates the disease. His claim is, however, that the disease is really a function of the animal that suffers, and not of the bacteria. An explosion of gunpowder is brought about by a spark, but everyone recognizes, of course, that the explosion is the function of the gunpowder, and not of the spark. Thus in regard to disease. Health is the result of the normal action of the body cells, and disease is the result of their abnormal action. It is hardly more correct to say that health is caused by the absence of bacteria, than to say that disease is caused by their presence. Bacteria may *provoke* abnormal action of the body cells, but disease itself is the result of this abnormal action. No one disease can appear in the body except one for which the body cells have a predisposition. Thus, in general, Hueppe places emphasis upon the functional activity of the body rather than upon the bacteria. Disease is the result of a number of factors, of which external conditions form one, the condition of the body another, and the presence of bacteria a third. If either one of the factors is lacking the disease does not occur.

As one reads this work of Hueppe it is evident that the author is trying to put himself quite decidedly in opposition to the school of Koch. But in reality there is less opposition than at first sight appears. The problem of infectious disease is a broad rather than a narrow subject. One side of the problem has been dwelt upon in extreme by the school of Koch. The other side of the matter has been too much neglected, and Dr. Hueppe is certainly right in calling further attention to it. The whole problem of dealing with infectious diseases is modified by this conception. Prevention of disease involves something more than avoiding bacteria. Hygienic measures need to do something more than try to destroy bacteria. Hueppe has little sympathy with the disinfecting mania which has in some places become so oppressive. In general, measures for combatting disease should be directed toward a general improvement in the conditions, rather than toward the destruction of specific bacteria.

Whatever we are inclined to think of these positions taken by the author, it is certain that he is right in emphasizing a side of the bacteriological problem that has been far too much neglected in the hurried search for specific bacteria which has occurred since Koch pointed out the way. All who desire to understand bacteriological problems should read this work carefully, if for no other reason than to avoid falling into the error of believing that our knowledge is greater than it is. Hueppe has certainly done a service in turning our attention to the fact that the physician should devote his energies to the man, rather than to the bacteria, and Professor Jordan has done a service to the advance of bacteriology by putting these suggestive chapters within reach of all American students.

H. W. CONN.



**Spirig, W.** Ueber die Diphtheriebacillen einer Hausepidemie. Zeitsch. f. Hyg., 30: 511-532, 1899.

This work covers investigations made on nine children from two different families in which there had been a diphtheria epidemic. Two were found to be free from infection, seven were infected. Four of these cases were infected with the classical Löffler bacillus. Two of the cases furnished cultures, and showed other characteristics which were more closely related to the pseudo-diphtheria bacillus than to Löffler's bacillus, and the ninth resembled quite closely the pseudo-diphtheria bacillus. The latter produced in animals paralysis identical with that produced by the classical Löffler bacillus. This latter property, combined with their evident common origin, points to variation in a specific bacillus. Differences in effect are to be explained by differences in predisposition at the time of infection. The results of these investigations may be briefly summed up as follows:

The epidemiological close connection of these cases of diphtheria furnish a test for the bacteriological differential diagnosis of the true and the false Löffler bacillus. The diphtheria bacillus may, in the individual cases of a diphtheria epidemic, show all stages of transition from the pseudo-diphtheria bacillus to the classical Löffler bacillus. The diphtheria bacillus may present all the characteristics of the pseudo-bacillus and still produce paralysis in animals. The specific serum reaction often fails in bacilli which possess all the characteristics of the pseudo-bacilli, give rise to paralysis, and epidemiologically belong to a single focus of true diphtheria infection. Neisser's granular staining, as well as the other differential diagnostic signs, is not absolutely certain.

H. H. W.

**Almqvist.** Ueber eine Methode das spezifische Gewicht von Bakterien und anderen Körperchen zu Bestimmen. Zeits. f. Hyg. u. Infektionskrankheiten. 28: 321-330, 1898. Rev. Centrbl. f. Bakt. 25: 619, 1899.

This is a method for determining the specific gravity of bacteria, etc., by centrifugation. For this purpose Almqvist uses a lactocrit with a steam turbine having eight thousand revolutions per minute. The medium containing the bacteria is placed in a tube constructed for the purpose, and centrifugated, with the result that the cells sink to the bottom. In a culture of hay bacteria, for example, there will be a layer of spores at the bottom, and above the rod-like organisms. The method may also be used to separate bacteria from the fluid containing them. Sodium iodide was found to be a good emulsion fluid. The hay bacillus centrifugated in this had a specific gravity of 1.4.

E. M. BRACE.

**Lunt.** On a Convenient Method of Preserving Living Pure Cultures of Water-Bacteria. Trans. Brit. Inst. Prevent. Med. I Ser. London, 1897. Rev. Hyg. Rundsch. 9: 238-239, 1899.

It was found that a variety of water bacteria — *B. fluorescens*, *violacens*, *iridescent*, *prodigiosus*, etc., could be cultivated in sterilized and in distilled water. They grew and reproduced in this medium for several years. The medium was not favorable for the growth of other forms, such as *B. coli*.

E. M. BRACE.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Harvard Medical School, Boston, Mass., to whom all books and papers  
on these subjects should be sent for review.

## CURRENT LITERATURE.

**Strekeisen.** Zur Lehre von der Fragmentatio Myocardi. Ziegler's Beitrage. 26: 105-130. 1899. The heart muscle was examined in 150 cases of death from disease, and in 18 of death from violence. Fresh razor sections mounted in salt solution and sections stained with carmine or with hæmatoxylin and eosin after hardening in Müller's fluid or in Formal-Müller, were studied.

Fragmentation occurred most frequently in the papillary muscles and wall of the left ventricle; next in the inter-ventricular septum, least often in the papillary muscles and trabeculæ of the right ventricle, and never in the auricles. The break was generally found through the cell body near the nucleus. Sometimes, however, it was through the cement substance. Associated fatty degeneration was seldom found, but pigment atrophy appeared to be a predisposing cause. Hæmorrhages were never observed, and the intercellular substance showed no abnormal changes.

In 150 cases, including tuberculosis, carcinoma, pneumonia, acute peritonitis, typhoid fever, and septicæmia, fragmentation occurred in 56.6 per cent. Two cases of tetanus showed very marked fragmentation.

As the result of his investigation the writer believes that fragmentation occurs just before death and is due to violent contraction of the heart muscle. The frequency of fragmentation in death from asphyxia supports this view. In death from acute infectious diseases, and after great efforts, he believes it very probable that the phenomena of asphyxia come on just before the fatal termination as the result of weakening of the heart and consequent diminished vascular pressure.

The absence of fragmentation in fatty degeneration of the heart muscle is due to the fact that the heart is so greatly weakened that contraction to the point of rupture and fragmentation of the fibers cannot take place. Pigment atrophy, on the other hand, leads to increased frangibility of the muscle elements.

The very frequent occurrence of fragmentation in cases of accidental death does not support the theory that a primary weakening of the heart muscle precedes fragmentation.

Although fragmentation occurs just before death, it is never an immediate cause of death.

A. M.

**Mitchell, L. J., and Le Count, E. R.** Report of a Necropsy in a Case of Acromegaly with a Critical Review of the Recorded Pathologic Anatomy. N. Y. Med. Jour., April 15th, 22d, and 29th, 1899.

The patient under observation was forty-three years old, 162.5 cm. in height, and weighed 81.1 kilogrammes.

The immediate cause of death was acute lobar pneumonia. At the post mortem examination the sella turcica was found to be enlarged; the hypophysis was a greyish red, softened, semi-fluid mass, not easily removed and not larger than normal. The thyroid body weighed 70

grams and appeared normal. The heart weighed 450, the liver 3530, the spleen 450, and the kidneys 300 grams.

*Anatomical Diagnosis.*—Lobar pneumonia, fibrinous pleuritis; cirrhosis of the liver; softening of hypophysis, enlargement of viscera.

Histological examination of the hypophysis showed the condition to be a hyperplasia. In the mesenteric lymph nodes, spleen, pancreas, adrenals, and thyroid an increase of connective tissue was observed.

A careful description, with measurements, of the cranium and long bones, is given.

The second part of the paper is a very complete résumé by Le Count concerning the pathological anatomy of acromegaly.

In concluding, the writer lays down the following propositions as those which have acquired the most prominence in the elucidation of the pathogenesis of acromegaly:

1. The cases of acromegaly associated with true tumor of the hypophysis are certainly not as numerous as has been heretofore supposed.

2. There is not as much constancy in the pathologic condition of the hypophysis as there is in an enlargement of the heart, thyroid gland, or the sella turcica.

3. Acromegaly does not depend, at least not solely, upon abolition of any function of the hypophysis.

4. A relationship between the thyroid gland and the hypophysis has been already proved.

5. It is not at all improbable that proliferation of the histological elements of the hypophysis may be instituted in some cases by a primary enlargement of the sella turcica; in other cases, an edema or hæmorrhage *ex vacuo*.

6. We have no reason for supposing that enlargement of the sella turcica may not be as constant an occurrence in acromegaly as the changes in other bones, or that it might not take place from similar cause or causes.

R. M. P.

**Schenck.** On Refractory Subcutaneous Abscesses Caused by a Fungus Possibly Related to the Sporotricha. Johns Hopkins Hosp. Bull. No. 93, 1898.

Schenck has isolated and studied a fungus pathogenic for man. The primary point of infection was on the index finger, whence it extended up

the radial side of the arm, following the lymph channels, and giving rise to several circumscribed indurations, which were in part broken down and ulcerated. Upon incising these areas, a quantity of gelatinous material was found from which he obtained the micro-organism in pure culture. It grew well on the ordinary media. Cover-slip preparations from agar and bouillon cultures showed two forms: (1) a thread-like branching form, or mycelium, and (2) oval, spore-like forms, or conidia.

The relation which the conidia bore to the mycelium was determined by observing their development in hanging drops of alkaline beef broth, which were placed under a moist bell-jar for forty-eight hours. The spores were quite commonly found, to the number of six or more, clustered to the tips of the spore-

bearing branches. The spores developed into unbranched germ tubes, and also into the branched mycelial forms.

Experiments upon dogs and mice proved the pathogenicity of the organism.

J. H. P.

**Warthin.** The Pathology of the Pacinian Corpuscle. Phila. Monthly Med. Jour., 1: 2. 1899.

The small amount of work which has been done on the pathology of these bodies is shown by the fact that only two references to previous work could be found in the literature.

The corpuscles studied were principally those found in the mesentery in the region of the pancreas.

In a case of perforation of the trachea by an œsophageal diverticulum, about 250 small hyaline, oval, or slightly flattened bodies, varying from the size of a pinhead to that of a small cherry, were found in the mesentery. The majority were transparent, jelly-like, and fluctuating. The larger ones appeared as small cysts with thin capsules. On rupture a thin mucilagenous fluid escaped. Microscopically there was seen a mucous-like degeneration of the tunic and core, congestion of blood vessels, small hæmorrhages, œdema of the capsule, and small cell infiltration in and about the capsule. This the writer believes to be an example of primary inflammation of the Pacinian corpuscle with marked myxomatous degeneration.

In a case of chronic parenchymatous nephritis, with general anasarca and marked ascites, hyaline bodies similar to those in the above case were found in the mesentery. Microscopically there was an increase of the inter-lamellar lymph, causing a wide separation of the lamellæ and increasing the bulk of the corpuscle. The tunic was thickened, the capsule and surrounding tissue œdematous; the capillaries congested. These local œdematous changes are to be considered as part of the general anasarca.

In two cases of chronic pulmonary tuberculosis, colloid and mucoid changes were found in the Pacinian corpuscles of the fascia about the seminal vesicles.

In corpuscles about thrombosed femoral vessels were found œdema, increase of interstitial tissue and vacuolization of the tunic, leucocytes in the lymph spaces, and thickening of the capsule. This is believed to be an example of chronic inflammation by direct extension.

In two cases in which histological examination showed the oviducts and ovaries removed at operation to be normal, in the Pacinian bodies of the mesentery were found myxomatous and colloid degeneration and deposition of lime salts.

In a case of hæmorrhagic pancreatitis with fat necrosis, the Pacinian bodies about the pancreas showed marked congestion, hæmorrhage, small round cell infiltration about nerve trunks, universal increase of leucocytes, and considerable liquefaction-necrosis. Thus the changes in these bodies may be inflammatory (acute and chronic), circulatory (congestion, œdema, hæmorrhage), and degenerative (mucoid, colloid, hyaline, calcification, necrosis).

The paper is illustrated by nine photo-micrographs, some of which, however, do not clearly represent the processes described.

R. M. P.



**Keifer.** Etiologie et développement des myomes de l'utérus. Belgique Société d'anatomie Pathologique. La Presse Medicale, No. 10, pp. 49, 1899.

muscle fibers, and which are afterwards penetrated by vessels which afford them nutrition. These are often found in the zone of tissue supplied by one vessel, or its collateral branches. In the center of these small myomata is often seen an arrangement of fibers, suggesting the lumen of a vessel in which the injection has not penetrated. Keifer believes that uterine myomata arise from these small muscular bodies. This takes place by hypertrophy of these bodies, as the result of their encysting vascular trunks rendered useless by thrombosis, prolonged compression, and other processes interfering with the circulation.

R. M. P.

**Ranvier.** Histologie de la peau. Définition et nomenclature des couches de l'épidermie chez l'homme et les mammifères. Acad. des Sci., Paris, La Presse Medicale, No. 7, pp. 32, 1899.

which rapid division takes place. 2. The filamentous layer, in which the cell protoplasm has many fibrils (prickle cells). 3. The granular layer, characterized by cells with eleidin granules. 4. The intermediate layer, which separates the two principal layers of the skin. 5. The stratum lucidum. 6. The stratum corneum. 7. The stratum disjunctum, or superficial desquamating layer. Each of these layers has perfectly distinct physical and chemical characteristics.

R. M. P.

**Ranvier.** Histologie de la peau. Sur quelques réactions histo-chimiques de l'eleidine. Acad. des Sci., Paris, La Presse Medicale, No. 12, pp. 55, 1899.

Eleidin is seen in the midst of the cells of the stratum granulosum in the form of granules. They stain readily with carmine, hæmatoxylin, and thionin.

Lime water does not dissolve them, but renders them more distinct by swelling the cellular protoplasm. In the transformation of the cells of the stratum granulosum to the stratum intermedium, the eleidin granules disappear, and there is found instead a homogeneous substance, staining still more strongly with carmine.

R. M. P.

**NEUTRAL RED.**—This is used as an *intra-vitam* stain, and reacts upon the cytoplasmic granules, and contents of mucus cells. Tadpoles placed in a  $\frac{1}{10000}$  or  $\frac{1}{100000}$  solution for a day or two are stained dark red.

Rothberger (Centrbl. f. Bakt. xxii, 515, 1898), uses it to distinguish Typhus from Coli bacilli. The concentrated aqueous solution is transparent, and has no trace of fluorescence, but a coli culture of neutral red agar after twenty-four hours becomes strongly fluorescent, and shows a clearing up of the stain, while a typhus culture remains unchanged. As far as the experiments were carried the reaction seemed to be characteristic for coli. The best preparation consists of 10 cc. fluid agar, three or four drops concentrated aqueous solution of neutral red, and about one-half cc. of a twenty-four hour coli bouillon. The fluorescence also shows well in gelatin cultures, but in these, large brownish-red crystals are formed. Other references: Zeits. f. wiss. Mik. xi, 2, 250, 1899. Galeotte, id. p. 193.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace, Biological Laboratory,  
University of Rochester, Rochester, N. Y.

**Thompson, Helen B.** The Total Number of Functional Nerve Cells in the Cerebral Cortex of Man, and the Percentage of the Total Volume of the Cortex composed of Nerve Cell Bodies, Calculated from Karl Hammarberg's Data; together with a Comparison of the Number of Giant Cells with the Number of Pyramidal Fibers. *Jour. Comp. Neur.* 9: 113-140, 2 figs., 1899.

estimates that the total number of functional nerve cells in the adult human cerebral cortex is 9,200 million that the proportion of functional nerve cells in the cortex is 1.37 per cent. and that the number of giant cells in the cerebral cortex of man is almost the same as the number of pyramidal fibers passing to the spinal cord.

E. M. B.

**Donaldson, H. H.** A Note on the Significance of the Small Volume of the Nerve Cell Bodies in the Cerebral Cortex of Man. *Jour. Comp. Neur.* 9: 141-149, 1899.

and that the cells of the cortex with their dendrons weigh about 13 g. The small volume of the cell bodies is a feature of the entire encephalon.

Comparison of human encephala grouped according to race, sex, mental power, stature, and age, show that differences in weight within each group are always more than twice that of all the nerve cell bodies, and hence these differences depend mainly upon variations in the medullary substance.

There is evidence that the dendrons of the cortical cells and their associated terminals furnish the structural basis for intelligence, and that degrees in mental power may be correlated with degrees in their complexity. The neurone may be divided into three parts, the receiving portion, the conducting, and the transmitting, corresponding to the cell body with its dendrons, the axone, and the ends of the branches, or the terminals. Since the weight of the cells of the encephalon is small, variations in the weight of the dendrons and terminals, which would be correlated with increased mental development, would be correspondingly slight, and though of the highest physiological importance, would not be detected by the method of weighing or might be masked by the greater growth of the medullary substance.

E. M. B.

**Hardesty, Irving.** The Number and Arrangement of the Fibers forming the Spinal Nerves of the Frog. (*Rana virescens*). *Jour. Comp. Neur.* 9: 64-112, pls. 6-13, 1899.

Data are added to those given by Karl Hammarberg in his monograph "Studien über Klinik und Pathologie der Idiotie nebst Untersuchungen über die normale Anatomie der Hirnrinde. Upsala, 1895." Basing her calculations upon these results, Miss Thompson esti-

From data given by Hammarberg and by Miss Thompson it is estimated that the weight of all the cell bodies in the human encephalon is less than 27 g.

and that the cells of the cortex with their dendrons weigh about 13 g. The

small volume of the cell bodies is a feature of the entire encephalon.

Comparison of human encephala grouped according to race, sex, mental power, stature, and age, show that differences in weight within each group are always more than twice that of all the nerve cell bodies, and hence these differences depend mainly upon variations in the medullary substance.

There is evidence that the dendrons of the cortical cells and their associated terminals furnish the structural basis for intelligence, and that degrees in mental power may be correlated with degrees in their complexity. The neurone may be divided into three parts, the receiving portion, the conducting, and the transmitting, corresponding to the cell body with its dendrons, the axone, and the ends of the branches, or the terminals. Since the weight of the cells of the encephalon is small, variations in the weight of the dendrons and terminals, which would be correlated with increased mental development, would be correspondingly slight, and though of the highest physiological importance, would not be detected by the method of weighing or might be masked by the greater growth of the medullary substance.

The spinal nerves vary in architecture according to their position, and the same nerves show variations of gross anatomy in different individuals.

By counting the nerve fibrils at different levels it was found that the number of fibrils in the ventral roots decreases from the spinal cord toward the ganglion,

while in the dorsal roots the number decreases from the ganglion toward the spinal cord. This decrease occurs among the small fibrils and is explained as being the natural result of the growth of nerve fibers.

In growing frogs the fibrils of the dorsal root increase more rapidly than those of the ventral root. Most of these fibrils go to innervate the skin and there is probably some relation between this increase and the increase in the surface of the skin.

There are more fibers in the trunk and dorsal branches than there are in both roots. This may be due to a splitting of the fibrils in the spinal ganglion, or there may be cells in the ganglion which send a process to the periphery but not to the centrum, or the distal branch of a T-shaped fiber may become medullated before the other branch, or there may be small medullated fibers from the sympathetic system which end in the ganglion.

*Technique.*—The cord and nerves were hardened *in situ* with osmic acid, washed, and then treated for one hour with a 5 per cent. aqueous solution of pyrogallol acid to emphasize the blackening. Cross sections of nerves were photographed, and when the fibrils were counted the section was compared with the photograph. A specially designed automatic registering machine was used for counting.

E. M. B.

**Döllken, A.** Weigert-Pal-Färbung sehr junger Gehirne. Zeit. f. wiss. Mikr., 15: 443-445, 1899.

Sections are cut about  $50\mu$  thick— $30\mu$  for rats or mice—and given the usual treatment for photoxylin plates after

Obregia. Stain in cold hematoxylin solution (Pal), for four or five days, then at  $37^{\circ}\text{C}$ . for two hours; after cooling leave in spring water for six or eight hours, without changing the water, and follow with alkaline distilled water (two or three drops of potassium to a liter) for fifteen minutes. Decolorize in about one-half per cent. potassium permanganate until the unmedullated parts begin to be transparent. Wash thoroughly in distilled water, place in a one per cent. solution of oxalic acid until the unmedullated parts become light brown, and the nuclei darker, and follow this with a quantity of distilled water in which the section will clear up still more. The fibers will be dark blue, the nuclei and cortex light brown to yellow, undeveloped places light yellow to white. Single steps should not be repeated. This is an especially delicate method for young brains, and any carelessness may result in over-bleaching the fibers, and in obscuring the contours of the ganglia and the nuclei.

The stain may be used after chromic acid fixation, but gives especially good results after a five or ten per cent. solution of formaldehyde. Material should be left in this until the potassium chromate will produce no further shrinking—at least fourteen days for a dog's brain, and from three to four weeks for a child's brain.

If chromic acid is used, from five to seven months are required. Thorough fixation and uniform hardening are of the greatest importance. The method demonstrates the form and arrangement of the ganglion cells as well as carmine, and also shows the path of the fibers.

E. M. B.

**Turner, John, M. B.** A Method of Examining Fresh Nerve Cells; with Notes Concerning their Structure and the Alterations caused in them by Disease. *Brain*, Winter No., 450-457, 1897.

and leave from three to twelve hours. When stained, remove the smallest portion possible with a scalpel and place on a slide in a drop of Farrant's solution, or water—it will keep longer in Farrant's—and cover with a cover-glass, then flatten the preparation under the microscope with two dissecting needles. Artificial light is best for studying these. These preparations cannot usually be preserved more than ten days, and are at their best a few days after mounting. E. M. B.

**Herrick-Coghill.** Note on the Methylen Blue Process. *Jour. Comp. Neur.* 8: 53, 1898.

In material impregnated with methylen blue, the stain tends to be extracted or to become diffuse during dehydration with alcohol previous to imbedding. To obviate this, C. L. Herrick recommends placing the object in glycerine, followed by a mixture of glycerine and gum arabic. After remaining in this mixture for a day, it is placed in a paper tray and the mixture allowed to evaporate until of a proper consistency for sectioning. E. M. B.

#### RECENT LITERATURE.

**Edinger, L.** The Anatomy of the Central Nervous System of Man and of Vertebrates in General. Trans. by W. S. Hall. 8vo, pp. XI + 446, illustrated. Philadelphia, 1899.

**Beddard, F. E.** Contribution to our Knowledge of the Cerebral Convolutions of the Gorilla. *Proc. Zool. Soc. London*, pp. 12, 7 figs., 1899.

**Cajal, S. R.** Estudios sobre la Corteza Cerebral humana. Madrid, *Rev. trim. microgr.* pp. 63, 23 gravures, 1899.

**Coghill, G. E.** Nerve Termini in the Skin of the Common Frog. Pt. 1. *Jour. Comp. Neur.* 9: 53-63, pls. 4-5, 1899.

## NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA McI. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

**Cusack, R.** Melting Points of Minerals. *Proc. R. Irish Acad.* 5: 399, 1897. *Abst. in Min. Mag.* 12: 49, 1898.

Apparatus by Joly consists essentially of a platinum ribbon which can be heated by an electric current. The temperature is obtained by micrometer measurements of the varying length of the ribbon. The substance in fine powder is placed on the ribbon and observed with a microscope, until the exact moment of fusion is reached. Readings can be obtained to within two degrees of temperature, and the method forms an easy way of determining minerals. The fusion points of forty common rock-forming minerals and ores are recorded.

Lord Kelvin (*Phil. Mag.* No. 1, 1899) has obtained different results in case of feldspar, mica, hornblende, and quartz.



**Goldschmidt, V.** Ueber Definition eines Zwillings. Zeit. f. Krystallographie. **30**: 254, 1898.

The author seeks to find a definition for a twin crystal which shall be satisfactory, *a.* genetically, expressing the manner of the combination of the embryonic particles, *b.* physically, expressing the arrangement of physically equivalent directions in the two individuals, and *c.* which shall finally express the arrangement of the equivalent faces of the two individuals. The definitions of the type: "An intergrowth such that individual I can be brought into position of individual II by rotation of 180 degrees around some axis," are rejected as genetically of no significance since such a rotation can not be assumed to have taken place, and since also some observed twins can not be explained by one rotation, but require the assumption of two. The definition chosen is: "A twin is a symmetrical intergrowth of two equivalent individual crystals." Or more briefly: "A twin is a symmetrical crystal pair." Under this definition, symmetrical combinations of right and left forms, such as occur in quartz, are not twins.

A. J. M.

**Viola, C.** Ueber Bestimmung und Isomorphismus der Feldspäthe. Zeit. f. Kryst. **30**: 232, 1898.

**Hankel, W. G.** Ueber die thermo-und piezo-Elektrischen Eigenschaften der Krystalle des ameisensauren Baryts, Bleioxyds, Strontians und Kalkes, des salpetersauren Baryts, und Bleioxyds des Schwefelsauren Kalis, des Glycocolls, Taurins und Quercits. Abhand. der math-phys. Classe der K. Sächs. Gesell. der Wissen. **24**: 475-496, 1898.

This is the twenty-first paper by Prof. Hankel, recording his studies of the electric charges developed by heat and pressure on different faces of crystals. The methods are described in earlier papers.

A. J. M.

**Goldschmidt, V.** Ueber Stereographische Projection. Zeit. f. Kryst. **30**: 260, 1898.

A description of a new and simple method for obtaining a stereographic projection from the position angles  $\varphi$  and  $\rho$  obtained in measuring with a two-circle goniometer. The writer, however, reaffirms the inferiority of the stereographic to the gnomonic projection for crystal work.

A. J. M.

**Stoeber, F.** Notice sur un appareil permettant de tailler un cristal suivant une direction déterminée et sur une méthode de taille des plaques aux faces parallèles. Bull. Ac. de Belgique, s. 3. **33**: 843, 1897.

Apparatus has the precision of the machines of Wülfing and Tutton, but is much simpler, being in fact a hand form of grinding apparatus. It depends

upon the fact that the position of a face is determined when its inclination to a given plane is known, and also the line in that plane through which the given face passes. The method of attaching the crystal so that any desired face or plane may be ground upon it is described.

L. McI. L.

#### INDIVIDUAL SPECIES.

**Cedarite.** R. Klebs. Jahrb. der K. preuss. Geol. Land. P. 1, 1896.

A new Canadian fossil resin, having composition C 78.15, H 9.89, S 0.31,

O 11.20, ash 0.45. It melts at 340 degrees.

**Lanarkite,** Reproduction artificielle. A. de Schulten. Bull. Soc. Min. **21**: 142, 1898.

Prepared by the action of basic acetate of lead.  $\text{Pb}(\text{C}_2\text{H}_3\text{O})_2$ , Pb O upon

sulphate of sodium. Comparison given between the artificial and natural crystals.

L. McI.

- Lorandit** von Allchar in Macedonien. V. Goldschmidt. Zeit. f. Kryst. **30**: 272-294, 1898. Careful crystallographic re-examination; Kenner's fourteen forms increased to thirty-two. L. McI. L.
- Mossite**, Vid. Skrift. Math. Nat. Klasse, Kristiania. No. 7, p. 1, 1887. Name given by Brögger to a niobotantalate of iron, found with yttrotantalite at Moss, Norway. Occurs in black tetragonal crystals, mostly twins, with twinning plane (101); may also be elongated parallel to (111), as with rutile.  $\epsilon=0.644$ . Composition =  $\text{Fe}(\text{Nb Ta})_2\text{O}_6$ , with Nb : Ta :: 1 : 1.  $G = 6.45$ . Author states that the mineral is near tapiolite, and that the crystals of tantalite (= skogbölite) represented in the text-books are practically identical with tapiolite. L. McI. L.
- Muscovite** compacte de Montrambert (Loire). G. Friedel. Bull. Soc. Min. **21**: 135, 1898. Occurs in the sericite schists of this locality, surrounding quartz nodules, in amorphous condition without cleavage. Brownish green in color and very translucent, with greasy lustre. Analysis corresponds exactly to formula for muscovite. H = a little less than calcite.  $G = 2.783$  at  $15^\circ\text{C}$ . Microscope proves it to be composed of fine fibres. Author considers it a variety of the sericite. L. McI. L.
- Quartz** — Pseudomorphs—Note on peculiar, found at Oweria Mine, Opitonui, North Island, New Zealand. G. H. F. Ulrich. Min. Mag. **12**: 33, 1898. Found near the surface in small auriferous quartz veins in the soft country rock. Probably owe their origin to infiltration of silica into cavities left by removal of calcite. The mass of the pseudomorph is compounded of small crystals of different orientation. L. McI. L.
- Prehnite** dans les roches. A. Lacroix. Bull. Soc. Min. **21**: 277, 1898. Author refers to his previous notes on determining zeolites and accompanying minerals (prehnite, etc.) and calls attention to the differences between prehnite and thomsonite. L. McI. L.
- Tourmaline**, The Quantitative Determination of Borac Acid in. Geo. W. Sargent. Thesis. Univ. of Penn. Philadelphia, 1898. In order to avoid the introduction of alkalies, shown to be objectionable, the author uses and describes in detail new methods for the decomposition of the mineral. The method is recommended which involves volatilization of the boric acid as the methyl ester and subsequent titration. Author concludes from his investigations that the formula proposed by Clarke probably represents the structure of tourmaline. But as yet such formulas have to be regarded with doubt, nothing being known regarding the magnitude of the molecule. L. McI. L.
- Wollastonite**, sur l'existence de la, comme élément d'une aplite. A. Lacroix. Bull. Soc. Min. **21**: 272, 1898. An unusual occurrence of wollastonite, as an essential rock-forming mineral, cited by author in a vein of aplite at the base of "Roc Blanc," at the bottom of the little valley of Barbonillères, (Ariège). L. McI. L.

## NEWS AND NOTES.

**TEMPORARY LABELLING OF GLASS SLIDES.**—It is often necessary in the microscopical examination of material, to attach a label to temporary preparations. Ordinary gummed paper has doubtless served for this purpose in most laboratories, as it has in ours, but selecting and attaching a proper piece often requires more time than a hurried worker likes to give.

Mr. W. A. Orton, formerly of the University of Vermont and now an assistant in our division, has in use a method which saves much time and much gummed paper, and is especially handy in the field. He has kindly permitted me to call it to the attention of those readers of the JOURNAL who may not already have in use a satisfactory method of temporary labelling. It is simply the grinding for half an inch or more of one or both ends of the slips. The grinding is quickly and easily done with a sand-blast. The ground surface will take pencil notes with the greatest clearness, and can be easily cleaned when desired. The same method of marking may be used for test-tubes. The slides which Mr. Orton has in use were ground for him by Professor Charles Jones of the Chemical Department of the University of Vermont, who perfected the sand-blast method of marking laboratory glassware.

ALBERT F. WOODS.

Division of Vegetable Physiology and Pathology, Department of Agriculture.

**ATTENDANCE AT MARINE BIOLOGICAL LABORATORY, WOODS HOLL, MASS., 1899.**—The Marine Biological Laboratory at Woods Holl, Mass., has just closed its twelfth annual session. The year has been a very successful one, additional courses were offered, attendance considerably increased, and a deep interest manifested. It is the purpose of the management to further broaden the scope of work. A thorough course in nature study will be introduced next year. To care for the increased numbers who are attending, it has become necessary to provide more room. An addition to the botany building and one new building for investigation laboratories are expected next year. We give herewith names of those in attendance this year:

*Embryology.*—Frank S. Bachelder, Univ. of Mich.; Cora J. Beckwith, Univ. of Mich.; Wm. C. Danforth, Northwestern Univ.; Ned Dearborn, N. H. Agri. College; Philip Dowell, Muhlenberg College; Ruth E. Eddy, Univ. of Minnesota; Mrs. John S. Ely, New Haven, Conn.; Adele M. Fielde, New York, N. Y.; Clarissa Fowler, Vassar College; George H. Garrey, Univ. of Chicago; Mary Greenman, Smith College; Maximilian Herzog, Chicago Polyclinic; F. M. M. Hull, Hamilton College; Clyde W. Jump, Univ. of Mich.; Christian P. Lommen, Univ. of S. Dakota; Ruth Marshall, Madison High School, Wis.; Alwin M. Pappenheimer, Harvard Univ.; Paul M. Rea, Williams College; Roy S. Richardson, Chicago Univ.; Augusta Rucker, Univ. of Texas; Richard H. Whitehead, Univ. of N. Carolina.

*Morphology.*—Robert C. Banes, Univ. of Penn.; Arthur M. Bean, Iowa College; Merton W. Bessey, Colby College; Laura Billings, Barnard College; Ella

M. Briggs, Mt. Holyoke College; Martha A. Brown, Aurora High School, Ill.; Gertrude S. Burlingham, Syracuse Univ.; Florence E. Clark, Mt. Holyoke College; Alice Stevens Davis, Mt. Holyoke College; Lida S. Eckel, Female Grammar School, Baltimore, Md.; Eugene H. Harper, Fargo College, Dakota; Mary E. Hart, Western College; Edith L. Hunter, Vassar College; Flora Isham, Breasley School, N. Y. City; Albert Keidel, Johns Hopkins Univ.; Wm. Erskine Kellicott, High School, Marysville, Ohio; Eleanor R. Kimball, Mt. Holyoke College; Rebecca Kite, W. Roxbury High School, Mass.; Maurice Lazenby, Johns Hopkins Univ.; Carrie E. Line, Illinois Woman's College; Helen E. Makepeace, Smith College; Clarence A. McCarthy, Univ. of Chicago; Anne G. Merritt, Smith College; Mary E. Morse, Woman's College, Baltimore; Henrietta F. Thacher, Bryn Mawr College; Hannah T. Rowley, Bryn Mawr College; Harriett S. Stockton, Smith College; H. D. Thompson, Moline, Illinois, High School; Jean D. Turner, Mt. Holyoke College; Elizabeth Fay Whitney, Smith College; Leonard W. Williams, Princeton Univ.; George H. Wilson, Wayne High School, Pa.

*Physiology.*—Samuel Bookman, Path. Institute, N. Y. City; Katharine B. Camp, Univ. of Chicago; Gaylord P. Clark, Syracuse Univ.; V. W. Sinthe, College of Medicine, Leipsic; Frank P. Knowlton, Syracuse Univ.; Charles G. Rogers, Syracuse Univ.; Annie Bell Sargent, Univ. of Pa.; Joseph S. Thomas, Princeton Univ.; Ralph W. Webster, Univ. of Chicago.

*Botany.*—Bradley M. Davis, Univ. of Chicago; George T. Moore, Dartmouth College; Mary A. Bowers, Smith College; Melville T. Cook, De Pauw Univ.; Julia T. Emerson, N. Y. City; Frances A. Hallock, Mt. Holyoke College; Anna Hoffman, Woman's College, Baltimore; Henrietta E. Hooker, Mt. Holyoke College; Ruth M. Huntington, Smith College; Walter S. Leathers, Univ. of Mississippi; Lillian J. MacRae, Franklin High School, Dorchester, Mass.; Charlotte A. Maynard, Roxbury High School, Mass.; Carolyn Morse, Wellesley College; Cora B. Mudge, Boston Public Schools; Henrietta Pratt, West Acton High School, Mass.; Margaret R. Putnam, Smith College; Winnifred J. Robinson, Univ. of Michigan; Anna M. Scringer, McGill Univ.; Fannie G. Smith, Smith College; Luella C. Whitney, Smith College; Daniel T. MacDougal, N. Y. Botanical Garden; David M. Mottier, Univ. of Indiana; Walter R. Shaw, Santa Rosa, Cal.; Dr. Erwin F. Smith, U. S. Dept. of Agriculture; Chas. O. Townsend, Maryland Agricultural College; Rodney H. True, Harvard University.

*Investigators.*—C. O. Whitman, Univ. of Chicago; Ulric Dahlgren, Princeton Univ.; E. B. Wilson, Columbia Univ.; Wm. Patten, Dartmouth College; M. M. Metcalf, Woman's College, Baltimore; Edwin G. Conklin, Univ. of Pennsylvania; Frank R. Lillie, Vassar College; A. L. Treadwell, Miami Univ.; George Lefevre, Univ. of Missouri; Henry E. Knowler, Johns Hopkins Univ.; Helen D. King, Bryn Mawr College; S. Emma Keith, Braintree, Mass.; Paul M. Jones, Vanderbilt Univ.; Geo. W. Hunter, Boys' High School, N. Y. City; Robert W. Hall, Yale Univ.; Walter E. Garrey, Univ. of Chicago; Edward G. Gardiner, Boston; Katharine Foot, Evanston, Ill.; Harry R. Fling, State Normal School, Oshkosh, Wis.; Virgil E. McCaskill, State Normal School, Stevens Point, Wis.; John V. Duyn, Syracuse Univ. Med. School; Winterton C. Curtis, Johns Hopkins Univ.; H. E. Crampton, Columbia Univ.; Alice W. Wilcox, Vassar College; Abbie H.



Turner, Mt. Holyoke College; Elizabeth W. Towle, Bryn Mawr College; John R. Murlin, Univ. of Pennsylvania; Louise B. Wallace, Smith College; Anne Moore, Vassar College; A. P. Hazen, Bryn Mawr College; Gary N. Calkins, Columbia Univ.; Cornelia M. Clapp, Mt. Holyoke College; Elliott R. Downing, Brooklyn, N. Y.; Martha Bunting, Columbia Univ.; Edward Ryneanson, High School, Pittsburg, Pa.; Mary Alice Wilcox, Wellesley College; Caroline B. Thompson, Univ. of Pennsylvania; Frederick S. Lee, Columbia Univ.; S. J. Holmes, Ontario, California; Robert M. Yerkes, Harvard Univ.; Edward Thorndike, Columbia Univ.; Oliver S. Strong, Columbia Univ.; E. C. Strobell, N. Y. City; George G. Scott, Williams College; Frederick C. Paulmier, Columbia Univ.; Wales H. Packard, Bradley Poly. Institute; W. W. Norman, Univ. of Texas; Margaret L. Nickerson, Univ. of Minnesota; A. D. Morrill, Hamilton College; T. H. Morgan, Bryn Mawr College; Thomas H. Montgomery, Univ. of Pennsylvania; Albert Mathews, Tufts Med. College; Elias P. Lyon, Bradley Polytechnic Institute; James H. McGregor, Columbia Univ.; Wm. A. Locy, Northwestern Univ.; Ralph S. Lillie, Chicago Univ.; Arthur E. Hunt, Manual Training High School, Brooklyn, N. Y.; Louise H. Snowden, Univ. of Pennsylvania.

THE LABORATORY OF THE UNIVERSITY OF THE PACIFIC.—The laboratory is arranged so that the light comes from one direction, it is heated with steam and has direct ventilation. Common tables, about 3 x 5, are placed two at a window, and one table is usually assigned to two students, each having his own private drawer. Microtome knives, scissors, scalpels, forceps, and other dissecting instruments, slides, cover-glasses, etc., are furnished by the student. There is a laboratory fee, sufficient to cover the expense of whatever course is chosen, and when this is paid the student is given the required apparatus, which may be returned at the end of the year, when the money will be refunded for all that is uninjured. Reagents and stains are also provided by the laboratory, each student being given a complete set of reagents that are in constant use, such as the different grades of alcohol, clearing fluids, etc. Although there are not as many microscopes as students, the work is arranged so that there are enough to supply all working at any one time. Responsibility for the care of instruments is placed upon the students as far as possible. Each student is required to keep a note-book, in which he records what he gets in the talks, keeps an accurate account of his work, and makes his drawings. The private biological laboratory has water, gas for lighting and heating, a full cabinet of materials, reagents and stains, a microtome and everything necessary for making slides. All specimens for preservation are usually prepared in this laboratory.

University of the Pacific. RUE D. FISH.

#### STAIN FOR NEUSSER'S PERINUCLEAR BASOPHILIC GRANULES.—

Saturated aqueous solution of	{ Acid fuchsin,	55 cc.
	{ Orange G,	70 cc.
	{ Methyl green,	80 cc.
Distilled water, . . . . .		150 cc.
Absolute alcohol, . . . . .		80 cc.
Glycerin, . . . . .		20 cc.

Film preparations of blood stained with this, show in gout, and leukæmia, a grouping of dark, blue-stained granules in uni- and multinucleated leucocytes. Cabot claims that these granules have no clinical significance, and states that he has a triple stain that brings out Neusser's granules in all blood, normal and abnormal.

# Journal of Applied Microscopy.

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## Formalin as a Preservative for Botanical Specimens.

The enormous strides that have been made in botanical teaching in the last few years have suggested opportunities for displaying material, by means of which many morphological, physiological, pathological, and ecological characteristics of plants may be shown. The older type of botanical museums, where a heterogeneous, unclassified mass of curious material is associated together, is of little importance other than that of a curiosity shop. To meet modern demands the collection need not, however, necessarily be a large one, but it should at least teach something and should be situated in the laboratory or lecture room, or close to it, where, if necessary, it may be easily made use of for purposes of illustration in the class room. In short, a collection of this nature constitutes a pedagogical collection, and it is much more valuable in aiding the modern methods of teaching botany than the primitive collections which many old botanical institutions now possess.

The writer, however, has a primitive collection at his disposal which has never been of any use in class work, and for this reason we have endeavored to build up a special collection for purpose of illustration in Morphology, Physiology, and Pathology, and as considerable attention has been given to preparing and mounting the specimens, a brief description may be of interest to botanists.

In the preparation of specimens we have made use of formalin, which, as a preservative, greatly excels any other solution as a means of displaying botanical specimens. The writer has used formalin in his laboratory for six years, for a great variety of purposes, with results that could not be obtained by the use of any other preservative known to him.

The strength of the formalin solution used for preserving specimens is four (4) parts of the forty per cent. solution to one hundred (100) parts of water. Two to three parts to one hundred have been tried, but solutions of these proportions have not proved satisfactory.

Most of our specimens have been kept in a 4-100 parts solution for five years without renewing, and with the exception of a slight tendency to form a precipitation in some of the jars, they are as clear as ever. The specimens are kept in both round and rectangular jars of glass, but we prefer the round jar of even texture and of good quality to any other, inasmuch as the slight magnifications of the specimen in such a jar we consider an advantage.

As a usual thing the material is preserved in formalin a few days before it is mounted up. This enables the coloring matter to become extracted, and when permanently mounted in the jar does not discolor the formalin.

When specimens are ready to mount, they are placed on a piece of ordinary glass cut so as to fit the jar which is to hold them. A piece of bibulus paper will take up the extraneous formalin adherent to the specimens; and after this has been done, a solution of gelatin, which hardens quickly upon cooling, is poured over and around the specimens until they are quite well embedded. After the gelatin has hardened, the specimens and their various parts may be labeled. We make use of printed labels, which are easily put on by covering them with a drop of transparent gelatin (see Figs. I, II, and III).

The specimens now mounted upon glass are ready to be placed in the jar of formalin, but in most instances we harden the gelatin still more; or, more properly speaking, dehydrate it by flooding the specimen for about five minutes with ninety-five per cent. alcohol. The gelatin is dehydrated and hardened still further when placed in the formalin.

When some opaque background is placed behind the specimens the gelatin is undiscernable. For the purpose of a background we use opaque black or white glass cut the same size as that upon which the specimens are mounted, although the specimens can be mounted directly upon opaque glass of any suitable color if desired.

Figures I and II show specimens put up in this manner, representing the morphology of the seed, and the more important stages of germination of the white lupine and sunflower. These specimens are representative of numerous types which we have prepared, and they can be made of considerable value in a laboratory of botany, especially in high schools where considerable attention is now paid to the morphology of seeds. When placed in a round glass jar with clear, clean glass, the details come out much more prominently than is shown in the photograph.

There is a considerable amount of material which can be mounted up and properly labeled, for illustrating features of especial interest in a modern botanical course. A large amount of pathological material might be advantageously shown by this method, inasmuch as in many instances the formalin does not affect the natural color pigments which are characteristic of many specimens. When the pigment is removed by formalin, as in the case of mushrooms, etc., the specimens can be painted with water colors, coated with gelatin, and the natural tints are thus retained. Waterproof India ink and most water colors are not affected by formalin. This method of preserving highly colored material has been used somewhat by the writer with successful results.

Another convenient method of preserving pathological material, especially diseased leaves, is by mounting them between two small plates of glass in glycerine formalin jelly, and covering the edges with paper to prevent evaporation, in much the same manner as lantern slides are mounted. This method retains the natural color of the specimens exceedingly well and is convenient for illustrating the various spot diseases of leaves (see Fig. IV).

Of especial value in teaching botany is the preserving and use for illustration



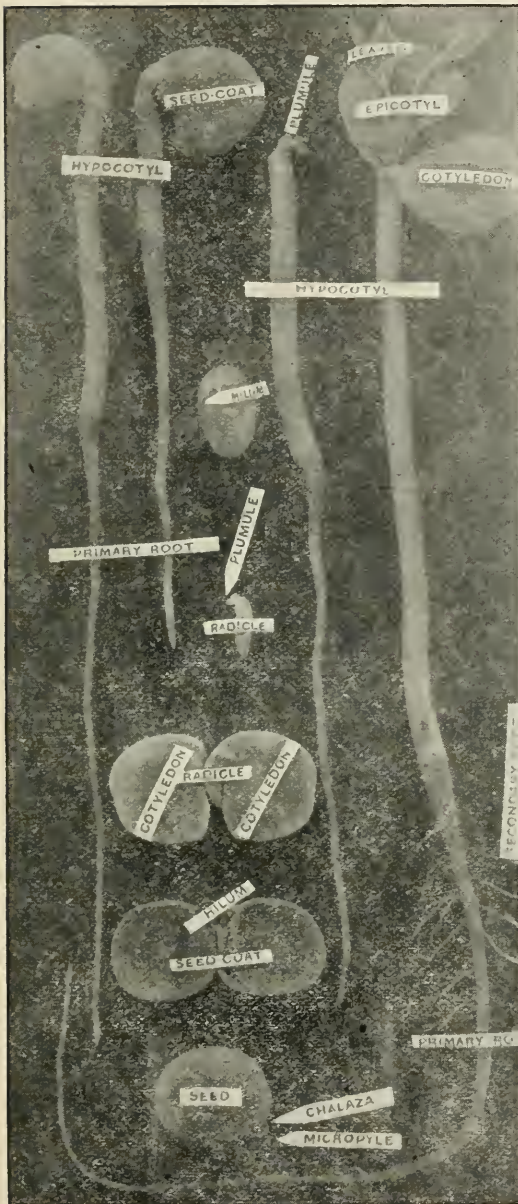


Figure I.

Representing the morphology of the White Lupine seed and more important stages of germination of the same.

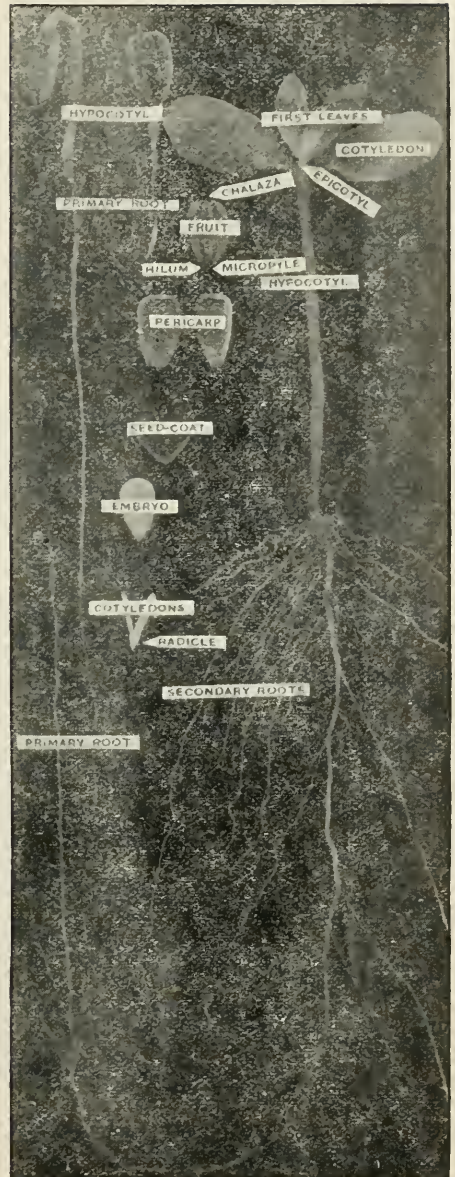


Figure II.

Representing the morphology of the Sunflower seed and more important stages of germination of the same.

of physiological material, such for example as: specimens of plants showing the effect of positive and negative heliotropism, geotropism, galvanotropism, rheotropism, hydrotropism, etc., also the effects of centrifugal force upon roots and stems; the regions of growth in length in roots and stems, various root tubercles and galls, reserve material in seeds, etc.



To illustrate the reserve material in seeds, one-half of the seed to be preserved is taken and placed in a solution of iodine. This, as is well known, will color the starch a deep blue color. Inasmuch as iodine is not a permanent stain, the color will soon fade when the substance is placed in formalin, but if a crystal of iodine is placed in the solution, the reaction can be retained indefinitely; or if a slight amount of nitric acid is placed in the formalin, the iodine color can be retained quite well for a long time. We have sections of seeds which have been in nitric acid formalin for three or more years, that completely retain their iodine reaction.

After having made extensive trials of both formalin and alcohol as media for preserving specimens, we are convinced that formalin is greatly superior to

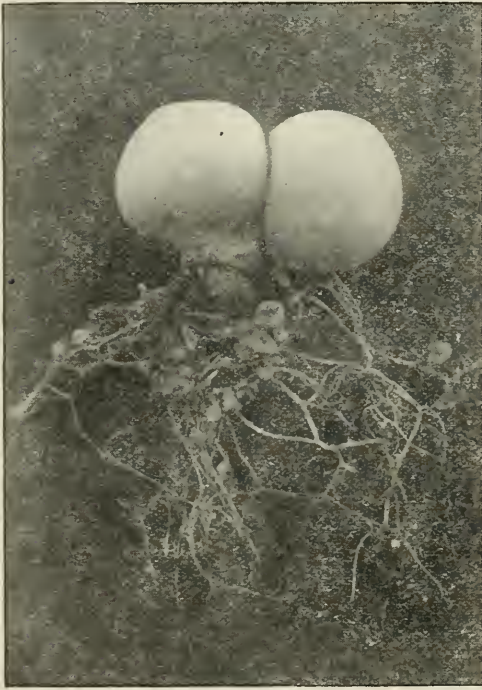


Figure III.  
Puff-ball. Mycelium development.



Figure IV.  
Leaf-spit of cherry mounted in formalin glycerine gelatin.

alcohol. Formalin solution gives a clean white color to tissues, whereas, alcohol always turns them to a dirty brown.

We found by repeated trials that it was impossible to preserve the delicate roots of the plants with alcohol and have them retain their natural color. Moreover, it was difficult, on account of the more volatile nature of alcohol, to keep specimens from shrivelling up when left out of the solutions for a few moments, as is necessary in mounting them up. With formalin, however, no such difficulty arises and the specimens may be left some time in exposure to the air without detriment.

G. E. STONE.

Botanical Laboratory, Massachusetts Agricultural College.

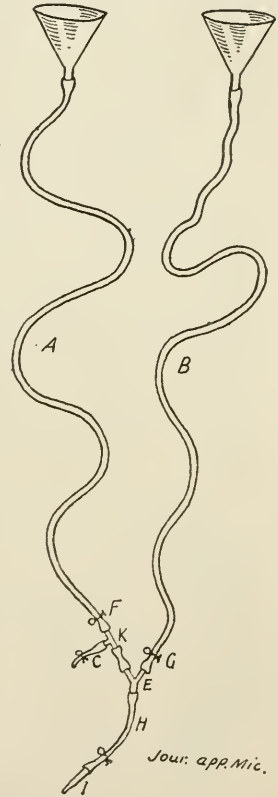
## Histological Fixation by Injection.

The necessity for fixing and preserving a large range of material for the courses in Comparative Histology in Leland Stanford Jr. University has led to the development of a simple method of in toto fixation, which has thus far most admirably answered every demand made upon it. For several years I have used this method upon all sorts of vertebrate material, such as Myxinoids (*Bdellostoma*), Selachians, Teleosts, Reptiles, Birds, and Mammals, and uniformly it has given the best of results. The ideal histological fixation would be to surround each individual cell with the killing liquid, and this can be practically obtained by injection and by injection only. The apparatus used must not be attacked by the fixing agents, and precautions must be taken to avoid such vascular contraction as would preclude the free access of the liquid to every part.

The apparatus in use here is extremely simple, consisting essentially of two glass funnels, two pieces of rubber tubing six or eight feet long, and two or three of shorter lengths, a Y glass tube, four pinch-cocks, and a supply of glass cannulae of various sizes.

The accompanying cut shows the arrangement of these parts. The long rubber tubes *A*, *B*, bearing funnels at their free ends, are joined together by the Y tube *E*, a simple pinch-cock being placed upon each immediately above *E*. The third arm of the Y tube is connected with a short rubber tube *H*, bearing the cannula *I*. Another pinch-cock, sometimes convenient, may be placed upon *H*. To facilitate the removal of bubbles of air a T tube, *K*, bearing a short rubber tube, *C*, with pinch-cock, should be placed near the lower end of the tube *A*.

The method of manipulation is extremely simple, varying but slightly for different animals. If, for example, a kitten is to be injected, the tube *B* is filled through its funnel with normal saline solution warmed to body temperature. The other funnel and tube *A* are filled with the fixing agent, *e. g.*, Zenker's fluid, warmed to the same degree. To the normal salt solution is added a few drops of lactic acid, amyl nitrite, or some similar reagent to insure dilation of the blood vessels and capillaries. The animal having been anæstheticised, the thorax is rapidly opened, the apex of the heart is cut across, and the cannula is introduced through the left ventricle into the ascending aorta and firmly tied. Before introducing the cannula the salt solution is allowed to run through the whole apparatus below the pinch-cock *F*, in order to avoid all bubbles of air. The pinch-cock on *H* is now opened and the salt solution is injected through the circulatory system, washing



out the blood before it, the necessary pressure being secured by raising the funnel to various heights as required. An injection of from thirty to forty seconds is sufficient to insure the thorough removal of the blood, which is indicated by its no longer escaping from the right ventricle. The pinch-cock *G* is now closed and *F* is opened, and the fixing fluid is injected in a similar manner. Practically every cell in the animal is thus instantly killed by direct contact with the fixing agent. The fixing fluid is allowed to run through the system for from five to ten minutes, after which the cannula is disconnected and further treatment adapted to the fixing agent employed is proceeded with. For example, if sublimate has been employed, the alimentary canal is opened and its contents removed, after which the animal is brought into alcohols of increasing strength, the body having been so opened as to permit free access of the alcohol to every part. Before or after the hardening has been completed by the alcohol, the various organs may be removed and placed in separate jars or bottles for further treatment and preservation. With Zenker's fluid, after injection is complete, the various organs are brought into a fresh supply of the fluid, where they remain over night, followed by washing out with water and graded alcohols.

By this method of fixation the exact normal relationships of the different parts are preserved, and pieces may be removed and sectioned with accuracy in any desired plane. For histological studies upon the nervous system, this method of *in toto* fixation gives the most admirable results, and thorough and equal hardening with the various bichromate mixtures is readily attained in a much shorter time than is usually the case. In addition, the removal of the brain and spinal cord is greatly facilitated by the firm consistency imparted to them.

By limiting the zone of action of the injection by ligating the arteries passing to other parts, any desired portion may be fixed alone. This applies with especial force to the brain and sense organs. The eye may be fixed *in situ*, permitting sections to be made showing the most delicate histological detail, without any of the usual vexatious collapsing. The internal ear also may be easily and thoroughly fixed by this method.

A number of useful modifications may readily suggest themselves to one making use of this apparatus as just described. One of the most convenient of these is the substitution of a glass three-way stop-cock for the Y tube and the pinch-cocks above it.

By substituting an isotonic solution containing no chlorides for the normal salt solution, and limiting the injection to the abdominal viscera, excellent preparations of endothelia may be secured. After the blood has been washed out and the abdominal cavity has been rinsed with the isotonic solution, a one-fourth to one per cent. solution of silver nitrate is injected. After an injection of from three to five minutes, the silver nitrate is washed out with distilled water, the mesentery is spread out and exposed to the sunlight until reduction is completed.

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Histological Laboratory, Leland Stanford Jr. University.



## METHODS IN PLANT HISTOLOGY.

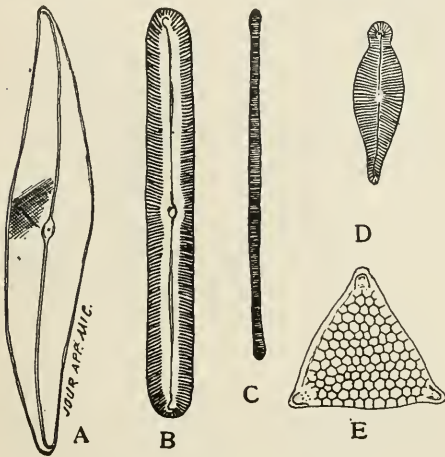
CHARLES J. CHAMBERLAIN.

## VIII.

## ALGAE.

*Diatoms.*—Diatoms and Desmids have been variously classified, and their position is not yet fully determined. A method for mounting fossil forms has

already been given. Living forms are often found clinging in great numbers to filamentous algæ, or forming gelatinous masses on various submerged plants. To obtain mounts of the frustules, use the method given in the March number of the JOURNAL. It is more difficult to get really good preparations showing the nucleus and chromatophores. If the diatoms are clinging to filamentous algæ, the algæ with the diatoms attached may be put into chromo-acetic acid (twenty-four hours), washed in water, stained, passed up through the alcohols, and cleared in xylol, or, better, in clove oil or bergamot oil, which do not dry up so rapidly. Here the diatoms may

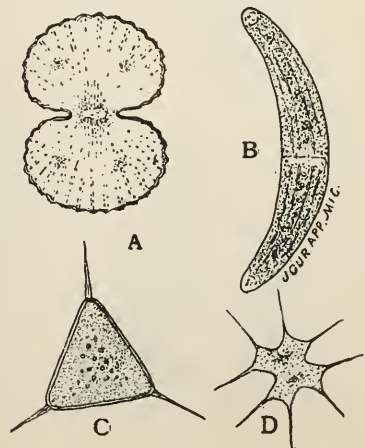
Fig. 18. Diatoms.  $\times 255$ .

A. *Pleurosigma angulatum*. B. *Navicula dactylis*. C. *Synedra biceps*. D. *Gomphonema sphaerophorum*. E. *Triceratium* sp.

be picked or scraped off from the other algæ, which will probably have become much shrunken by this treatment. Mount in balsam. Haidenhain's iron alum hæmatoxylin is recommended for the nucleus and the centrosome, which is quite prominent in diatoms. Delafield's hæmatoxylin and erythrosin give a good view of the nucleus and chromatophore. If a glycerine mount is preferred, the iron alum hæmatoxylin is a good stain.

When the material is in gelatinous masses, it may be fixed in chromo-acetic acid and imbedded in paraffin. There will, of course, be some difficulty in cutting, and many frustules will be broken, but there will, nevertheless, be occasional views which show details better than when the diatoms are mounted whole.

*Desmids.*—When these forms are very abundant they may be treated like the fila-

Fig. 19. Desmids.  $\times 255$ .

From glycerine preparations. Not stained. A. *Cosmarium pectinoides*. B. *Closterium cucumis*. C. *Staurostrum cornutum*. D. *Arthrodesmus octocornis*.



mentous algæ, except that extreme care must be taken while changing fluids. It often happens that a single desirable desmid appears when examining field collections. In such a case, remove it with a fine pipette, and get it into a drop of water on a clean slide, invert it over a bottle of one per cent. osmic acid for a minute, leave the slide exposed to the air until the water has almost all evaporated, and then add a drop of ten per cent. glycerine. In a few hours (6-24), put on a cover and seal. It requires more time, care, and patience than it is worth to attempt staining in such a case.

#### PHAEOPHYCEAE.

The brown algæ are almost exclusively marine. The slime, so prevalent in the group, often makes the technique difficult.

*Ectocarpus*.—Fix in chromo-acetic acid (twenty-four hours), wash in fresh water, since the salt of sea water may cause inconvenience in subsequent processes. Stock material should be passed up to seventy

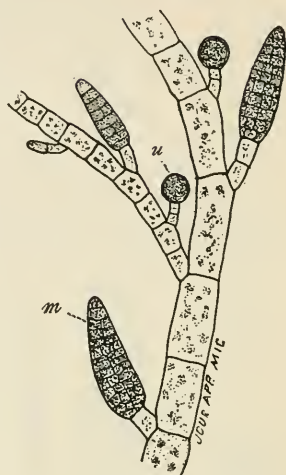


Fig. 20. *Ectocarpus confervoides*. From a preparation stained in Mayer's hæmalum, and mounted in glycerine.  $\times 255$ . m, Multilocular sporangium, u, Unilocular sporangium.

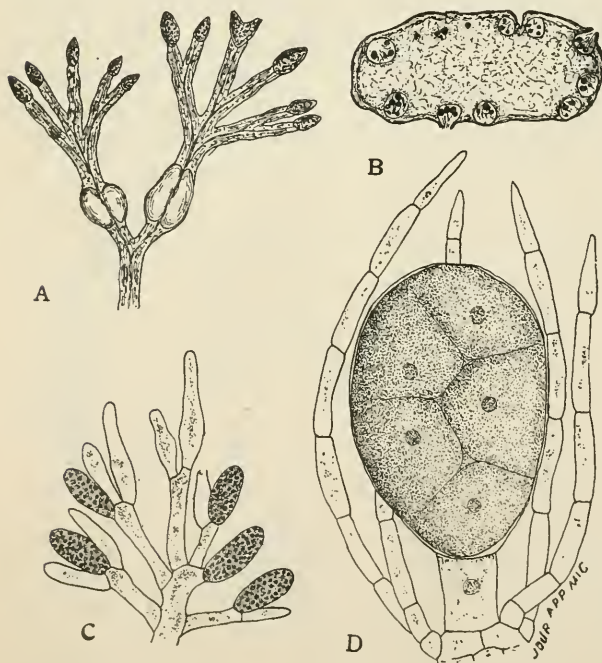


Fig. 21. *Fucus vesiculosus*.

A, Small portion of plant showing bladders and fruiting branches. One-half natural size. B, Transverse section of fruiting branch showing oogonial conceptacles.  $\times 6$ . C, Antheridia and paraphyses. From a preparation fixed in chromo-acetic acid, stained in borax carmine, teased out and mounted in balsam.  $\times 255$ . D, Oogonium showing five of the eight oospores. Prepared as in C.

per cent. alcohol for safe keeping. Eosin or Mayer's hæmalum are good for glycerine mounts. If paraffin sections are to be made, the material must be brought very gradually from absolute alcohol into the clearing agent, and from the clearing agent into the paraffin.

Other filamentous members of the group, as well as the more delicate membranous forms, may be treated like *Ectocarpus*.

*Fucus*.—*Fucus* may be fixed, washed, and preserved like *Ectocarpus*. It is difficult to get paraffin sections across the whole fertile branch, but elegant sections may be obtained by cutting narrow strips containing

a few conceptacles. The safranin-gentian-violet-orange combination is good for such sections. For such views as are represented in C and D the material should be stained in bulk in borax carmine or alum carmine. The process for borax carmine is as follows:

- a. Borax carmine, 24 hours.
- b. Acid alcohol (1 per cent. HCl in 70 per cent. alcohol), 10 minutes.
- c. 70 to 100 per cent. alcohol, 2 hours each.
- d. Clear in cedar oil, bergamot oil, or oil of cloves.
- e. Tease out the contents of the conceptacles sufficiently to show details, and mount in balsam.

The process for alum carmine is the same, except that no acid alcohol is used.

Sections like that shown in B are easily cut in celloidin. After staining in borax carmine or alum carmine, imbed in celloidin in the usual way. After hardening the celloidin in chloroform, put the block into 95 per cent. alcohol for fifteen or twenty minutes, and then into Eycleshymer's clearing fluid (equal parts bergamot oil, cedar oil, and carbolic acid), until thoroughly cleared. The block may be left here indefinitely, and sections may at any time be mounted in balsam as soon as they are cut.

*Chorda*, *Laminaria*, and similar forms may be treated like *Fucus*.

#### RHODOPHYCEAE.

The red algæ belong almost exclusively to salt water, but a few genera are found only in fresh water, usually running water, and a few forms occur both in salt and in fresh water.

The technique is more difficult than in the case of the brown algæ. Until something better is suggested the same method of fixing and washing may be used as for the brown algæ. Picric acid, corrosive sublimate, and absolute alcohol have been tried, but do not give as good results as the chromo-acetic acid or Flemming's fluid.

*Batrachospermum*.—This is a green, fresh water member of the red algæ. It is not very uncommon in small streams.

The cells are so small that it is hardly worth while to attempt sectioning them. Very good preparations showing the nuclei may be obtained by staining in Mayer's hæmalum, or Haidenhain's iron alum hæmatoxylin. After the material is in glycerine ready for

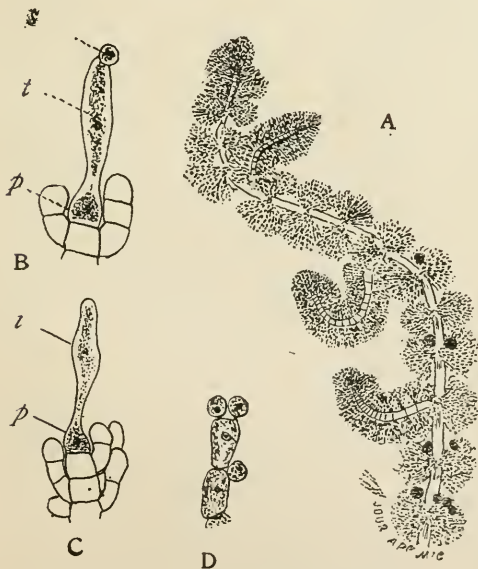


Fig. 22. *Batrachospermum moniliforme*.

From a preparation stained in Mayer's hæmalum and mounted in glycerine. A. Portion of plant showing branches and several cystocarps.  $\times 25$ . B. A procarpic branch showing carpegonium (p), and trichogyne (t), with an antherozoid (s) attached.  $\times 255$ . C. A younger branch showing carpegonium and trichogyne.  $\times 255$ . D. Branch with three antherozoids.  $\times 255$ .

mounting, tease out a small portion and still further dissociate the filaments by tapping smartly on the cover.

Material stained in eosin shows the external structure well, but may not bring out the nuclei.

*Polysiphonia*. — For preparations like those shown in the figure, eosin is a very good stain. To get a brilliant coloring, stain for about twenty-four hours, so that the one per cent. acetic acid may be allowed to act for several minutes without making the stain look weak. Wash thoroughly in water. Not the slightest trace of color should be allowed to come out in the glycerine.

Sections showing the central and peripheral siphons and other gross features are easily cut in celloidin. It is not very difficult to cut paraffin sections, but the nuclei are so small and so hard to bring out that such preparations had better be left for the specialist.

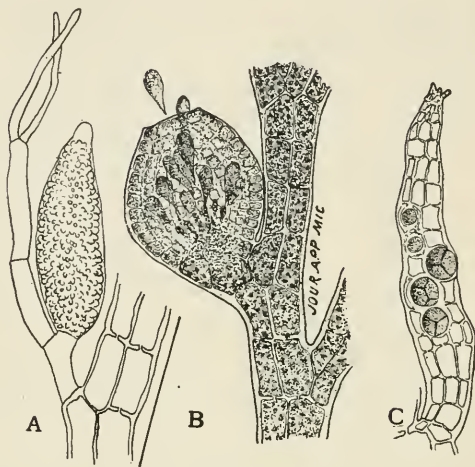


Fig. 23. *Polysiphonia fibrillosa*.

From a preparation fixed in chromo-acetic acid, stained in eosin, and mounted in glycerine.  $\times 255$ . A. An antheridium. B. A cystocarp with carpospores. C. Tetrasporic branch with tetraspores.

## FUNGI.

### SCHIZOMYCETES.

*Bacteria*.—No attempt will be made to give methods here. The larger coccus, bacillus, and spirillum forms may be brought out by the method already indicated. Fine preparations may be obtained by inoculating a mouse with *Anthrax* or some other form, and then cutting paraffin sections of favorable organs. Gentian-violet with a faint bismark brown for a background makes a good combination.

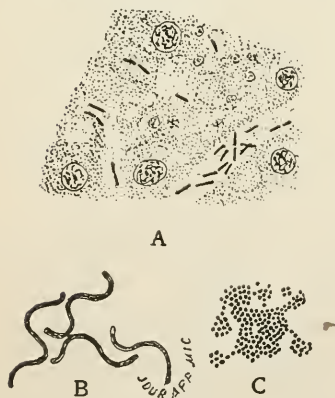


Fig. 24. *Bacteria*.  $\times 535$ .

A. *Bacillus anthracis*, from a paraffin section cut from the liver of a mouse. Fixed in chromo-acetic acid, stained in methyl-violet and bismark brown, and mounted in balsam. B. *Staphylococcus pyogenes aureus*. From a preparation stained in gentian violet. C. *Spirillum* sp. From a preparation stained in fuchsin.

*Leptothrix* may often be obtained by scraping the inside of the cheek. *Beggiatoa*, a form with oscillating movements like *Oscillaria*, is often found in foul water. Its presence may be indicated by whitish patches on the bottom.

It is doubtful whether the bacteria possess even a morphological forerunner of the nucleus of higher plants, consequently there need be no disappointment if the larger forms, like some of the *Beggiatoas*, fail to show a nucleus.



## MYXOMYCETES.

With the exception of a few forms like *Fuligo* (often found on oak stumps and on oak bark in tan yards), the Myxomycetes are small, and are usually overlooked by collectors. A careful examination of rotting logs in moist woods will usually reveal an abundance of these delicate and beautiful organisms. They should be pinned to the bottom of a box for safe carrying. For herbarium specimens they are simply allowed to dry, and are then fastened with glue or paste to the bottom of a small box.

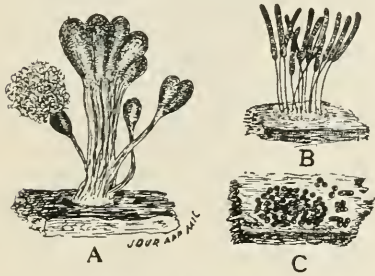


Fig. 25. Myxomycetes.

Growing on rotten wood. A. *Hemitrichia rubiformis*,  $\times 20$ . B. *Stemonitis ferruginea*. Natural size. C. *Trichia varia*,  $\times 1\frac{1}{2}$ .

recommended. Excellent methods for living cultures were given in the January and February (1898) numbers of the JOURNAL.

## PHYCOMYCETES.

*Mucor*.—This familiar mould appears with great regularity on bread. The following is a sure and rapid method for obtaining *Mucor*: Place a glass tumbler in a plate of water, put a slice of bread on the tumbler, and cover with a glass jar.

To obtain such a series as is shown in A-D of the figure, the material should be studied before the sporangia begin to turn black. The phase in the life history indicated in F-H is rarely seen. The writer would be glad to hear from any who have met this phase, especially if the information could be accompanied by a few dry zygospores.

Corrosive sublimate (4 per cent.) in 50 per cent. alcohol, used hot, may be recommended as a fixing agent. Hæmalum, or Delafield's hæmatoxylin, are good for glycerine preparations.

A very satisfactory study may be made from the living material.

*Cystopus*.—This fungus is quite common on Cruciferæ, where the white "blisters" or "white rust" form quite conspicuous patches.

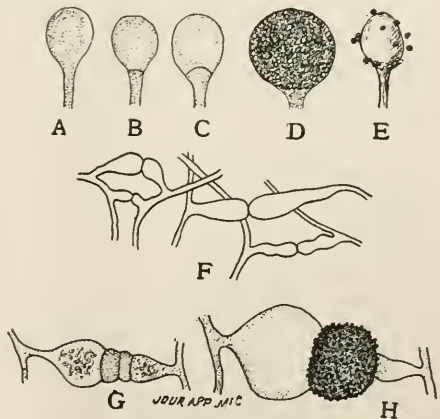


Fig. 26. *Mucor stolonifer*.  $\times 255$ .

A-D. Successive stages in the development of the sporangium. Drawn from living material. E. Columella with a few spores adhering. F-H. Stages in the formation of the zygospore. From a preparation fixed in corrosive sublimate, stained in Delafield's hæmatoxylin, and mounted in glycerine.



Affected portions of leaves and stems should be fixed in chromo-acetic acid and cut in paraffin. Safranin-gentian violet-orange seems to be the best stain for differentiating the nuclei.

It is more difficult to get good sections of the plant in the oösporic condition. The oösporic phase of *Cystopus bliti* is easily recognized on *Amarantus*, where the oöspores may be seen with the naked eye by holding the leaf up to the light. While better nuclear staining can be secured with chromic or Flemming material, it will be found somewhat easier to cut material which has been fixed in picric acid (1 per cent. solution in 70 per cent. alcohol). Celloidin sections, stained in Delafield's hæmatoxylin, can be recommended for showing the position of oögonia and antheridia, although such sections are too thick to give satisfactory views of the nuclei.

(To be continued.)

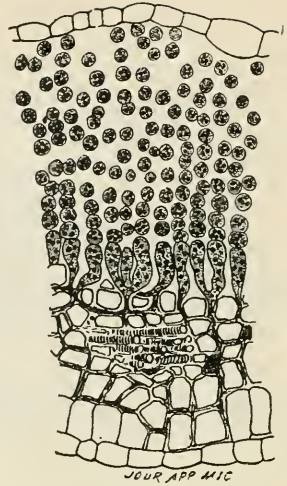


Fig. 27. *Cystopus candidus* on Capsella.

Trans. sec. of a blister on the leaf.  $\times 255$ . From a preparation fixed in Flemming's fluid and stained in safranin-gentian violet-orange.

## A Convenient Method of Numbering Slides in a Series.

In cutting sections in series, their number is often so great as to run over a considerable number of slides. It is well, therefore, to designate the slides in some way which will not be affected by the reagents through which they are passed. A convenient method is as follows: After the sections have been placed on the slides with the fixative beneath, number them with a pencil 1, 2, 3, 4, etc., on the ground glass edge at the end of the slide. As this edge is too narrow for ordinary figures, make as many little lines across it as there are units in the number. If this is larger than five, a space might be left between each group of five marks to aid in reading them. This method may also be used to show upon which side the sections are. For instance, if when the slide is held in the hand the sections are on the upper side, and the numbers are placed on the end, nearer the left-hand corner, the sections will always be above when the slide is held in the same relative position again. The numbering of slides in this way is very easily done, and the writer has found common pencil-marks to be unaffected by the ordinary reagents used in sections.

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## An Apparatus and Method for Preparing Agar.

In the April, 1898, number of this JOURNAL, Miss Marion H. Carter, of Cornell University, published a method for preparing agar for the cultivation of bacteria. Being impressed with its simplicity, I determined to give it a trial, inasmuch as it promised to save considerable labor. Repeated trials have proved that much of the drudgery connected with the preparation of this important medium may be avoided by employing Miss Carter's method.

The object of the present article is to emphasize this fact, and to describe an apparatus which I have devised for employing the method.

The apparatus consists mainly of an ordinary nine-inch copper funnel, with an inch-wide perpendicular wall around the top, which may be obtained ready-made at the shops. To it are added a cover, and three legs having a spread equal to the greatest diameter of the funnel. The spout may be closed by means of a rubber stopper, or a cap may be soldered on. In the former case, the funnel will be found useful in other directions. A funnel of this size will hold a gallon, and gives ample room for work.

If one liter of medium is wanted, place about 900cc. of bouillon in the funnel, add ten or fifteen grams, of pulverized agar, according as a 1 per cent. or a 1½ per cent. solution is desired, to the other 100cc., and mix thoroughly by rubbing up in a mortar or a beaker. Place the mixture thus made in the funnel and stir thoroughly. If the agar is in the stalk form, it may be put directly into the funnel after having been cut up. Now place the funnel with its contents in a 11x13-inch can, containing sufficient water to last for two hours, and boil. An Arnold sterilizer may be used instead of the can, if desired. After boiling for an hour, turn off the gas, remove the covers, and stir the agar solution, especially that in the spout of the funnel. Replace the covers, and boil another hour. Turn off the gas, and allow the whole to cool, taking care not to disturb the solution at this time. After solidification has taken place, remove the funnel, invert it on a glass plate, and the agar will come away as a mould. That portion from the spout will be found made up of the insoluble materials which ordinarily interfere with filtration, and which usually amount to about 1 per cent. of the whole mass. It is to be sliced off and thrown away. The remaining portion will be sufficiently clear for ordinary purposes, and may at once be remelted, distributed, and sterilized. Should it not be sufficiently clear, it may be clarified by the usual method. Filtration may then be readily accomplished, since most of the foreign and insoluble matter has been removed by the sedimentation process.

As might be supposed, the nutritive qualities of agar thus made are in no way interfered with. Over fifty different species of bacteria have been grown upon it with perfect success, by the writer.

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Washington, D. C.

## A Convenient Micro-Polariscope for Food Examination.

The value of the micro-polariscope as a means of distinguishing starch granules from other bodies and starch granules of different plants from each other, does not appear to be fully recognized by some of the leading authorities on the microscopy of foods, chief dependence being placed on the iodine test,

the shape and size of the granules, the form and location of the hilum, and the character of the concentric rings.

While it is true that the skilled microscopist can distinguish the various starches when illuminated by ordinary light, still if he is provided with a suitably arranged polariscope, he can often reach his conclusions, with a saving of both time and eyesight.

Among the authors who advocate the use of this apparatus may be mentioned Tripe, Blythe, Richardson, and McGill.

The classification of starches adopted by Blythe<sup>1</sup> is based on the appearance of the granules with polarized light and the selenite plate. Richardson<sup>2</sup> illustrates the application of the polariscope in the examination, with reference to adulteration, of ground spices and condiments. McGill<sup>3</sup> describes its use in the quantitative determination of wheat flour in ginger.

The writer has found polarized light of special service in the examination of sausage for potato,

wheat, and maize flour, which are frequently added to this food as carriers of water. If the fat is not removed before examination, the colorless starch granules are not readily distinguished from the fat globules, and the fat also interferes with the iodine test, but with crossed prisms the starch not only becomes evident, but the particular variety present may be readily determined.

One of the chief drawbacks to the use of this appliance is that biological

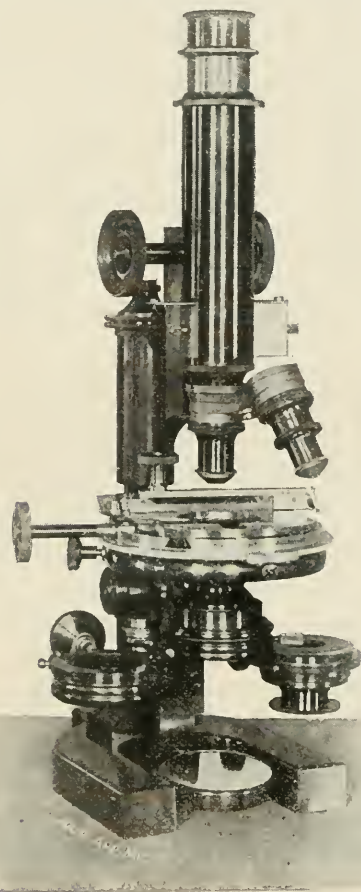


Fig. 1.

1. Foods. Composition and Analysis. Fourth Edition, p. 170.

2. U. S. Dept. of Agriculture, Div. Chem., Bull. 13, Part II.

3. This journal, Vol. 1, p. 51.

microscopes are not usually arranged for changing quickly from plain to polarized light, and *vice versa*. Usually the polarizer is fitted to the substage ring, which carries the Abbe condenser and cannot be attached until the substage is lowered and the condenser is removed. For attaching the analyzer the microscope tube must be raised, the objective or nose-piece removed, and the analyzer screwed at the upper end to the tube, and at the lower end to the objective or nose-piece. Both the substage and the tube must be readjusted before the object can be viewed. The change back again to plain illumination is equally laborious. These operations not only consume several minutes each time the polariscope is brought into service, but also, if often repeated, are ruinous to the screw threads and other parts of the apparatus.

In Fig. 1 is shown a Bausch & Lomb Continental microscope fitted with an arrangement devised by the writer, which obviates all the disadvantages which have been named. The polarizer (Fig. 2) is carried on an arm below the substage, and swings into position from the right, at the same time forcing the iris diaphragm out of position to the left. The analyzer is mounted in the same manner as in petrographical instruments, the prism being contained in a box which slides in the main tube, so that when pushed to the right the light passes through the prism, but when pushed to the left, a round opening permits unobstructed vision.

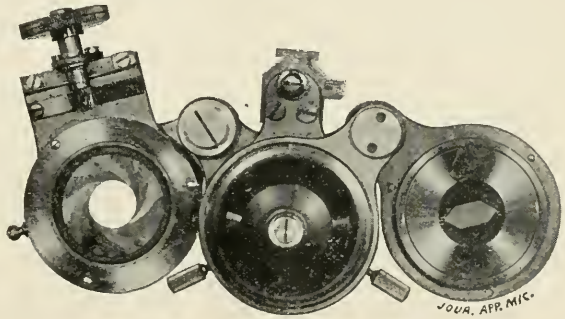


Fig. 2.

When polarized light is desired, the polarizer is pushed to the left, and the analyzer box to the right, and the change to plain illumination is accomplished by the reverse operations. Either change can be made in less than a second, without disturbing the adjustment either of the tube or the substage, and without damage to the instrument.

Another advantage is that the condenser may be used in conjunction with the polarizer, thus rendering the crosses on wheat, rye, barley, and some other feebly active starches much more distinct.

Selenite plates are mounted in a metal slip and are used on the stage.

Now that the microscopic examination of food products is coming into importance in the United States, the question arises as to the form of microscope best adapted for this work.

After experience with several types of instruments, the writer prefers a Continental stand, provided with two-thirds and one-sixth-inch objectives, double nose-piece, one and two-inch oculars, micrometer mounted in extra one-inch ocular, mechanical stage, iris diaphragm, Abbé condenser, and the polarizing apparatus which has been described.

A microscope of this description (Fig. 1) has been in constant use at this station for several years and has been found to fully meet the somewhat peculiar requirements of the food analyst.

A. L. WINTON.

Connecticut Agricultural Experiment Station.



## New Laboratory Apparatus.

### I. AN IMPROVISED HYDROGEN GENERATOR.

Almost every laboratory makes certain modifications or adaptations of well known pieces of apparatus, formulæ, and methods, such changes being often convenient for conditions under which they are applied. Very often these changes would not be improvements under other conditions, and not uncommonly the individual who devised them regards the changes trifling in character, and presumes that, of course, some one else has similarly applied the idea involved, and that it might be presumptuous on his part to publish so simple a thing. The pieces of apparatus to be described, we have no doubt, have been

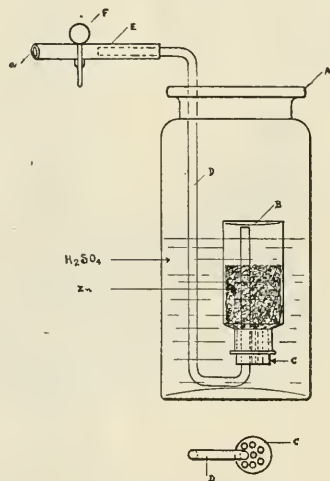


Figure I. An Improved Hydrogen Generator.

(Drawn by P. A. Sheaff.)

- A. Container filled with sulphuric acid up to the level shown. B. Eight to sixteen-ounce bottle turned upside down and closed by perforated stopper shown at C. Through this perforated stopper passes the short arm of the conducting tube D. It will be observed that the end of the tube within the bottle B extends above the acid level. Just below the large drawing is an end view of the stopper showing perforations, and the conducting tube D passing through the stopper. The horizontal arm of the conducting tube D is connected with a large piece of rubber tubing, E, and the flow of gas controlled by a Mohr's pinch-cock, F. Any piece of apparatus may be connected to the rubber tubing at a. If the connecting tube D be bent in the shape of an S at the point where the letter D is shown, this S may be hooked over the top of the container to hold the bottle B out of the fluid when not in use.

utilized by other workers, in one form or another, but we are unfamiliar with any publication which has been made on the subject.

During the progress of some work on the bacillus of tetanus, there was constant need of a hydrogen generator which could be thoroughly controlled, and at the same time one which could be depended upon, under all conditions, to afford sufficient hydrogen for any anaërobic apparatus in use. Of the many forms of hydrogen generator, none seemed at the time available, and so the apparatus shown in Fig. 1 was, after some experiment, devised by Dr. Coplin. It can be constructed anywhere and under almost all conditions. All that is needed is a bottle, preferably a salt mouth bottle of from eight to sixteen ounces capacity (bottle shown at B in illustration). A stopper with perforation, or cor-

rugated at the side by cutting out pieces, is a convenience, but not a necessity. Such a stopper may be of cork or wood, or, what is best, of rubber. It should fit with sufficient tightness to prevent the submerged bottle from coming loose, spilling the zinc, and rising as a result of the buoyancy of the contained gas. A glass or rubber tube (such as shown in the illustration at D) is passed through a hole in the cork so that the end within the bottle reaches to the bottom of the bottle, B; the length of the projecting end of the tube will depend upon the depth of the outside container, and the distance to which it will be desired to conduct the gas to other apparatus. It is best to make this tube of glass, although rubber tubing will do; where fragility is an objection, lead might be substituted, although the authors have never used anything but glass. The conducting tube, D, should have an internal diameter of .5 to 1 cm., and should be sufficiently heavy not to break easily. The outside container, A, may be a wooden bucket, crockery jar, ordinary battery jar, or Whitall-Tatum museum jar, with a perforation in the lid through which the tube can be passed; a large sized Millville gas jar, such as made by Whitall-Tatum, will be found most convenient. We used such a jar for some time, but it was accidentally broken, and since that time we have used an ordinary museum jar; when the apparatus is not in use we lift out the bottle containing the zinc and set it to one side, and stopper the jar containing the sulphuric acid. To use the apparatus, a sufficient quantity of scrap zinc, or zinc turnings, is placed in the bottle, B, the bottle is then laid upon its side to permit of the insertion of the tube and stopper. The bottle is now turned upside down as in the illustration, and inserted into the diluted sulphuric acid in the outside container. The height of the liquid in the outside container should be so adjusted that when the bottle containing the zinc is pushed down as far in the sulphuric acid as it will go (and the acid permitted to rise to its own level within the bottle containing the zinc), the acid will not come nearer than two cm. of the end of the tube within the bottle, B. The object of this precaution is, of course, apparent. Should the acid be admitted sufficiently near the bottom of the bottle (which is now the top) it would run over into the tube and form a trap at the bottom, or if the tube is small it might be carried on into the apparatus with which the tube is connected. At the external end of the tube a piece of thick rubber tubing, E, is attached, and a Mohr's pinch-cock, F, is adjusted so as to collapse it. The rubber tubing may be attached to anaërobic culture apparatus of any kind, or to any other piece of apparatus which it may be desired to use. If, at any time, the exit of the gas from the tube be prevented, the acid is forced away from the zinc by the continued generation of gas, and in a very short time chemic action is arrested. The apparatus may be used for the generation of sulphuretted hydrogen, or, with slight modification, of carbon-dioxid, etc.

The points which have recommended the apparatus to us are: (1) its simplicity, (2) its cheapness, (3) any student can make such apparatus from materials always available, (4) it may, by some of the suggestions already given, be kept practically always ready for use, (5) it is not easily broken, and all the parts may be replaced quickly and cheaply, (6) it is efficient.

## II. SIMPLE APPARATUS FOR FILTERING THROUGH A PASTEUR-CHAMBERLAND FILTER.

The accompanying diagram explains this apparatus better than anything which could be added in the text. For use, the chimney is simply grasped by a good retort clamp, or large size burette holder, so as to steady it and hold it firmly in place. The top of the filtering bougie is connected as usual with the flask, in which a vacuum can be made if desired. The bougie, with its connections, may be sterilized, as may the lamp chimney. After sterilization the rubber tubing, CC, which may have been disinfected in some chemical solution, or by steam, is slipped over the top of the chimney,

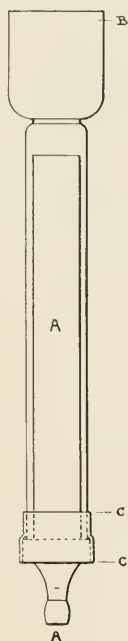


Figure II. Simple Apparatus for Filtration through Pasteur-Chamberland Filter.

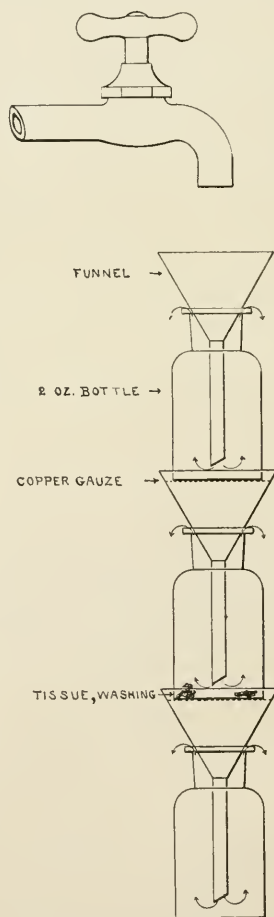
(Drawn by P. A. Sheaff.)

B. A chimney of the ordinary German student's lamp. Chimney as shown is inverted. AA. Pasteur-Chamberland bougie, upon the shoulder of which the chimney rests. CC. Piece of heavy rubber tubing which slips over the small end of the chimney and the shoulder of the bougie.

comparatively rapidly. We are of course perfectly aware of more elaborate forms of apparatus involving the same principle, but we recommend this by reason of its simplicity, and the fact that it may be improvised almost anywhere.

## III. BATTERY OF JARS FOR WASHING SPECIMENS.

In some form or another the principle illustrated in the accompanying drawing has been utilized in our laboratories for a number of years. Large specimens, such as lungs, are



— DIAGRAM OF BATTERY OF BOTTLES FOR WASHING SPECIMENS IN FLOWING WATER. ARROWS SHOW COURSE OF WATER —  
Scale = 1/4-Inch.

Figure III. Battery of Jars for Washing Specimens.

(Drawn by P. A. Sheaff.)

washed in large jars. Under such circumstances a large funnel will be needed, usually with a wide angle. If the neck of the funnel is not long enough to go to the bottom of the jar, a piece of rubber tubing is attached. If the buoyancy of the specimen is such that it floats or shifts the funnel, a piece of brick or a whole brick, or some other equally weighty object, is placed in the funnel. Very few bottles or jars will be found in which the flange of the funnel will fit sufficiently close to preclude the constant flow of a medium sized stream of water. Of course, should a bottle be found fitting so very tightly, it will only be necessary to place a little piece of wire or string between the flange of the funnel and the neck of the bottle. The wire gauze shown for raising one bottle upon the funnel beneath, is not strictly necessary; in fact we have used it but very little, usually finding that the bottle will readily keep its place in the funnel, and will permit water to flow by it to continue the washing of the bottles beneath. The quantity of water flowing through the jars is determined by the size of the opening in the funnel; any excess simply flows over the edge of the funnel and mixes with the water, which has taken the course indicated by the arrows. One great advantage of the method is that, no matter what the head of water may be, high or low, or how irregular and inconstant the flow, no damage is likely to occur to the specimen. All bottles which depend upon siphonage for changing the water are open to certain objections. For example, if for any reason inflow is arrested just at the time siphonage is occurring, the specimen may be left with an insufficient quantity of water upon it. Where apparatus for washing requires direct connection with the water supply, sudden alterations in pressure or occasional back suction (siphonal action) may lead to disastrous results.

Where the water supply may contain particles of dirt, we sometimes tie a bunch of absorbent cotton over the mouth of the spigot; where a Pasteur or other efficient filter is attached to the spigot, this is not necessary. If the bottles are numbered or otherwise marked by a wax pencil, the markings will not rub off, or if the label is simply pasted on, and a thin layer of paraffin rubbed over it, there will be no trouble during the washing process. The same principle can be applied for washing sections cemented on the slide, and placed in a Jefferson staining jar.

*Remarks.*—As stated in the beginning of the paper, we have no doubt that similar appliances have been utilized by, possibly, a number of workers; we claim no originality, and simply say that no search has been made through the literature for descriptions of anything analogous. All of the appliances above described have been used for a number of years, and have given satisfaction.

W. M. L. COPLIN, M. D., Director.

P. A. SHEAFF, Undergraduate Assistant.

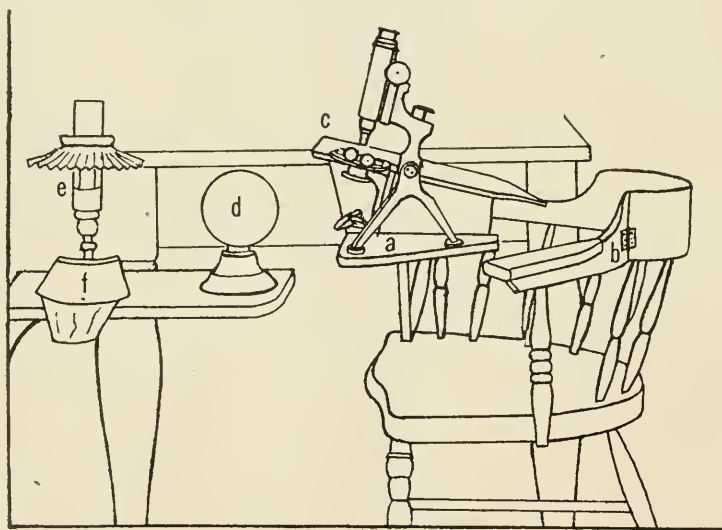
Laboratories of the Jefferson Medical College Hospital.



## Some Helpful Laboratory Apparatus.

In the opinion of the writer, no conveniences are too elaborate for the microscopist who daily spends several hours of exacting labor in the minute and careful observation of details under high-power lenses. Any appliances that give ease to eyes or body not only render the work less arduous, but increase the efficiency of the investigator's powers of observation.

Realizing this, the writer has, by degrees, accumulated a number of contrivances that, according to his experience, combine to render observation with the microscope easier and more precise. These different pieces of apparatus may not be new to many, but possibly an account of their combination into a working series may suggest to others schemes for greater ease in manipulation, and thus prove of some value. In this hope, a description of the installation is here given.



Explanation of figure: *a*, shelf for microscope; *b*, swinging arm of chair; *c*, inclined drawing surface; *d*, balloon flask condenser; *e*, Welsbach light; *f*, eye shade with cloth attached.

The principal feature of it is a chair so modified as to carry a microscope and a drawing board in positions that will require the least strain upon the body and arms. In constructing it, a large chair with the right arm broadened into a slanting writing surface was employed. The hard-wood writing top was removed, and in its stead was placed a soft pine board of similar size and shape. Below this, at a distance equal to the height of the microscope stage above the table, was fastened a horizontal shelf just large enough to receive the microscope foot. This is sufficiently distant from the seat of the chair to allow the right leg of the observer to rest beneath it. This arrangement places the microscope nearly enough in front of the operator to render access to the eyepiece easy and convenient. At the same time this projection makes it difficult to enter the

chair, and accordingly the left arm was cut away and suspended on a hinge so that it might afford a free entrance to the chair and yet be in a condition to be swung back, to serve as a rest for the left arm of the operator. All the wood-work was then painted black in order to do away with disturbing reflections.

When it is desired to use the apparatus, the observer places the microscope on the shelf provided for that purpose, and inclines it until the stage becomes level with and parallel to the drawing surface. He then swings out the left arm of the chair, seats himself, and returns the swinging arm to its closed position. Thus situated, he finds both arms supported at about the level of the elbows and the ocular of the microscope at a suitable height for comfortable observation. When the camera lucida is employed, the image is traced upon paper fastened to the pine drawing board by long, stout pins thrust into the soft wood. The right arm, meanwhile, rests in a comfortable, natural position, and the hand supports and guides the pencil as in writing. The accompanying cut makes the various details of construction and operation plain.

*Illumination.*—Ordinary sunlight is, in many ways, unsatisfactory. For accurate comparative work, a more reliable and manageable source of light becomes almost necessary. The writer has found the Welsbach incandescent gas lamp eminently suited to the requirements of the microscopist. A very excellent method of using it is to cast an image of the glowing mantle upon the mirror by means of a balloon flask filled with ammonio-cupric sulphate solution. The flask acts as a condensing lens, and by mutually arranging it and the lamp, the proper sized mantle image may be thrown upon the mirror. A turned wooden base, bearing a hole in the center for the reception of the flask neck, has proven the most satisfactory mounting, although improvised ones in the form of tumblers or empty fruit jars have often served the purpose.

The final character of the light is entirely dependent upon the solution in the flask. This must be of such a nature that it will give a nearly white light without the yellow of the gas-light or the blue of an excess of the ammonio-cupric salt appearing. With the most careful adjustment, however, the light has a cold bluish cast that obscures some details in sections stained by the iron-hæmatoxylin method. The writer found by experiment that this difficulty could be overcome and a beautiful soft white light secured by adding one or two drops of a saturated alcoholic solution of safranin to the liter flask of properly diluted ammonio-cupric sulphate solution. It has been found necessary to renew the safranin every week or two, since it fades upon exposure. The light thus modified leaves nothing to be desired, and has served in the most delicate work with high powers.

In connection with the chair previously described, a shelf is used for holding the gas lamp and the flask condenser. This projects from the wall, somewhat below the level of the microscope base, and with the bottom of the incandescent mantle at a height of seven inches from the table, needs to be from twenty to twenty-four inches long to permit the proper adjustments.

*Protection for the Eyes.*—Almost as important as the illumination of the object is the prevention of extraneous light from entering the eyes of the observer. Nothing is more trying to the visual organs or more inimical to accurate obser-

vation than a confusion of lights reflected from half a hundred polished surfaces, supplemented by a general glare from wall, ceiling, and floor. One needs only to protect the eyes for a short time from all light except that coming through the ocular in order to prove the facts stated. With the eyes shaded it will be found that very much less illumination will be required, while at the same time definition of a much higher order will be secured.

To profit by these obvious advantages, the writer adopted the simple device of wearing an eye-shade to which had been fastened a square of black cloth at the upper edge. This cloth is of such a length that when the lower edge is gathered around the top of the microscope tube and the shade adjusted on the head, the eye will be about low enough for observation. The cloth falls against the face and around the eyes so that all light except that coming from the object is prevented from entering. Thus protected, the eyes can be used continuously for hours without becoming fatigued, and the vision is clear and distinct. I believe that no one who has once employed some such means of protecting the eyes will ever conduct further serious observations without thus conserving his eyesight.

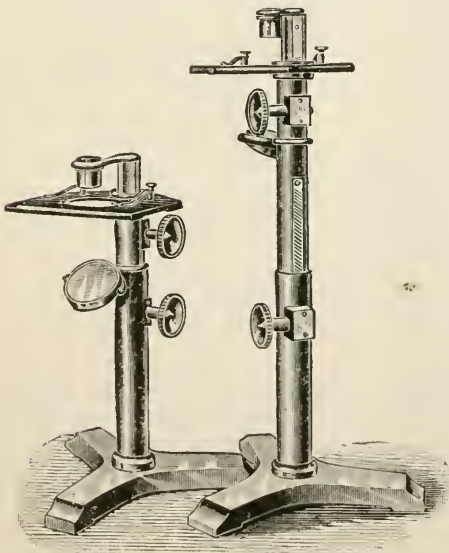
C. E. McCLUNG.

University of Kansas.

### An Adjustable Dissecting Microscope.

The accompanying photograph shows two dissecting microscopes, both of the same pattern. Each is provided with two racks and pinions; one for adjusting the lens, the other, lower one, for raising and lowering the mirror, stage, and lens.

In the one shown at the left, the lens when in focus is 260 mm. from the table, about the average height of compound microscopes. In the one shown



at the right, the lens is 350 mm. from the table. The lower rack and pinion permits of all intermediate heights according to the needs of the operator. The instrument weighs four pounds and five ounces, which is sufficient to give it stability, even when racked up to the highest point, and has nickel finish, giving it a fine appearance which is in no way a deception.

The idea of having a dissecting microscope of this kind was suggested to me from having been obliged to either double myself into a position to see an object through the common makes of low dissecting microscopes, or being obliged to place them on temporary supports which generally proved unsteady. With this instrument one can work for a long time comfortably, because he is in a comfortable position, and his arms and hands take a natural position.

One dozen of these were made by special order for use in the botanical laboratory of this institution, and are, as far as I know, the only instruments of the kind in existence. They have been in use two years, along with equally good instruments of the low form. Students are allowed to choose which they will use, and the tall form instruments are always chosen first by the average student, only the student of very low stature thinking the low form will serve him better.

The lenses were screwed in only sufficiently to hold them firmly, but the students soon found out they were removable, so it was found more satisfactory to screw the lenses into the arm so they could not be removed with the hands.

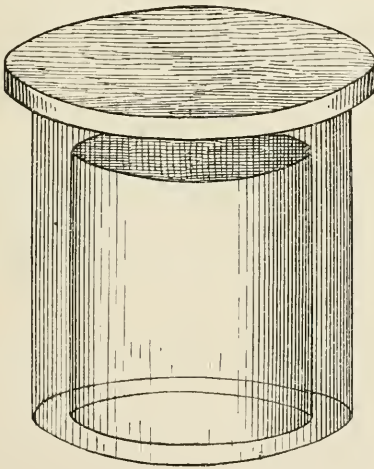
E. E. BOGUE.

Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma.

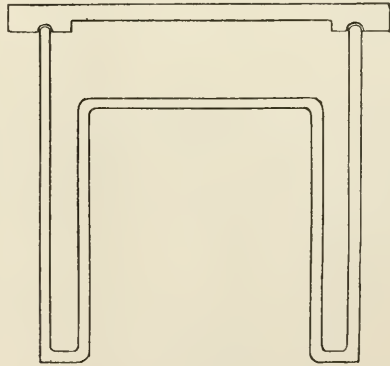
### A Design for a Convenient Staining Dish.

Since microscopic technique is usually rather tedious, it is desirable to have all apparatus so constructed that it will give the least trouble and care to the investigator. The following design, shown in the figures, is presented as a model for a convenient staining dish. It combines the advantages of the Stender dish and the staining dish, usually made by placing a number of crystallizing dishes inside of each other.

The dish should be made of clear white glass two millimeters thick, with straight, vertical walls and with the top surface accurately ground into a groove in the cover. The cover should be six millimeters thick around the edge. The



1



2

dimensions are as follows: The inside height is eighty millimeters; height of central part, sixty millimeters; internal diameter, eighty millimeters; diameter of central column, sixty-eight millimeters.

One of the most important points about a staining dish is that it is not easily upset; another, that only a small amount of the reagent is necessary. The dish described above meets these requirements and is large enough to hold from eight to ten slides at a time. The slides can be transferred with great ease, the only precaution necessary is to place them so that the sections are turned outward.

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Botanical Dept., Ohio State University.



# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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GENERALLY speaking, it is difficult to determine the value of a publication to its readers for the reason that, beyond an occasional letter of praise from "an old subscriber" or "an admirer" who is influenced by some particular article that coincides with his own views, editors and publishers rarely receive any comments on their work.

The reader who finds the publication of interest continues it, while another, interested in a different field, throws the copies aside as received, and at the expiration of his subscription orders it discontinued. At this point the publisher receives the first hint that his publication is lacking in something. But what?

It is this lack of consultation and exchange of ideas between the publisher and his patrons which is responsible to a large extent for the deficiencies which the public find in the publication. Most publishers are glad to be set right when drifting into impractical fields, and to add new subjects when required. Of none is this more true than of the JOURNAL, and it is with the hope of bringing ourselves more in touch with our readers, and thus knowing better how to provide for their particular requirements, that we would earnestly request an expression from each one who has found need for a kind of material not already provided.

\* \* \*

It has often happened that, in visiting the various laboratories, complaints have been made regarding the ineffectiveness of the laboratory equipment, and upon investigation it was found that what was originally, and should still be, a very excellent equipment, had materially degenerated through the lack of proper daily care.

In large laboratories it is almost impossible for the professor or instructor to give time to the personal inspection and care of apparatus, but it rarely happens that a student assistant can not be found competent to perform all that is required. For example, the touch of a perspiring finger on the lacquer of an instrument has little effect if removed the same day, but left to itself produces an indelible stain. The few specks of dust which fall upon the eyepiece during the day may be of little consequence, but the accumulation of weeks will render the best glass useless.

The sliding brass parts of the microscope can not be protected by lacquer, and in the course of time an accumulation of verdegris, small though it may be, on the surfaces of a delicately fitted adjustment will destroy its fine working, while if wiped away daily or weekly the instrument will retain its delicacy indefinitely. Next to the system of making each student responsible for every piece of apparatus he uses, frequent and systematic inspection is most essential to the maintenance of a satisfactory working equipment.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Lang, W. H.** The Prothallus of *Lycopodium* clavatum. Ann. Bot. 13: 279-317, pl. 16-17, 1899.

So little is known about the prothallia of *Lycopodium* that the present paper, although based upon only half a dozen specimens, constitutes a valuable contribution. The smallest prothallium was 4 mm. in length, by 3 mm. in breadth, and about 0.5 mm. thick. The color was a dirty white. The largest prothallium was about twice as long, with other dimensions in proportion. In general appearance these prothallia recall those of *Botrychium virginianum* as described by Jeffreys. A vertical section shows on the under side of the prothallium a layer of colorless cells, above which are several layers of cells infested by an endophytic fungus. The cells of the upper half of the prothallium are smaller, and are entirely free from the fungus. The antheridium is developed from a single superficial cell, and in the mature condition does not project above the surface. The archegonium, which is also developed from a single superficial cell, projects considerably above the surface. There does not seem to be a basal cell. Within the archegonium are the oosphere and six or eight canal cells, the lowest presumably the ventral canal cell.

The value of the prothallium as a taxonomic character is discussed at some length, and the writer concludes that species of *Lycopodium* which possess similar saprophytic prothallia should not, on that ground alone, be regarded as closely related. The prothallium of *Botrychium virginianum*, in form, texture, endophytic fungus, position of sexual organs, and subterranean saprophytic habit, resembles the prothallia of *Lycopodium*, but the resemblance merely shows how an appearance of genetic relationship may result from modifications due to a similar subterranean saprophytic habit.

C. J. C.

**Fischer, Dr. Alfred.** Fixirung, Färbung, und Bau des Protoplasmas. Kritische Untersuchungen über Technik und Theorie in der neueren Zellforschung. Pp. X+362. One colored plate and 21 text figures. Jena, Gustav Fischer. 11 Marks.

This book brings together into convenient form an immense amount of scattered literature relating to the fixing, staining, and structure of protoplasm.

Part I, dealing with fixing agents, considers in detail the solutions in common use, and describes their action upon the various cell contents as peptone, proalbumose, nucleic acid, nuclein, etc., etc. The numerous experiments with substances of known chemical composition should be of value in determining what are to be regarded as artefacts, and what as structural elements of the tissues. The closing chapter on the fixation of cell contents will be found especially helpful by those engaged in cytological work.

Part II (128 pages) is devoted to staining. Both theory and practice are

considered in detail. Some of the topics are: the washing out of the fixing agent and its significance in theories of staining; staining in simple staining solutions without differentiation; double staining with simple solutions; simultaneous double staining with mixed stains; impregnation; objections to the physical theory of staining; chromatin, and the fundamental doctrines of staining. Here again experiments upon substances of known chemical composition occupy a large part of the space.

Part III deals with the structure of protoplasm. Spindles, centrosomes, and radiations are thoroughly discussed, and artificial figures are compared with those occurring normally. Chromatin is treated in the paragraphs on granules. The various theories of the structure of protoplasm, as the granula theory, the network theory, the filar theory, and the foam structure theory, are critically reviewed.

Investigations upon the structure of protoplasm demand not only extreme skill in mechanical manipulation, but also a knowledge of the principles underlying fixing, staining, and other details of micro-technique. This book puts the whole subject of micro-technique upon a firmer and more philosophical basis, and gives an up-to-date discussion of modern theories of protoplasmic structure.

C. J. C.

**Atkinson, G. F.** Studies on Reduction in Plants.  
Bot. Gaz. 28: 1-26, pl. 1-6, 1899.

In *Ariscema triphyllum* the writer finds that a qualitative reduction of the chromatin takes place during the first mitosis in the pollen mother cells. *Ariscema* differs from forms hitherto described, in that both a longitudinal and a transverse division of the chromosomes takes place during the first mitosis in the pollen mother cell. Korschelt's work on the annelid *Ophryotrocha* is quoted as an instance of a reducing division during the first mitosis, and Belajeff's work on *Iris* is cited as a case of reducing division during the second mitosis in the pollen mother cell. Calkin's work on *Pteris* and *Adiantum* is also quoted as an example of a reducing division in plants.

In *Trillium grandiflorum* there is also a reduction division, but it occurs at the second mitosis in the pollen mother cell. The author suggests that the explanation of the divergent and often contradictory accounts of various investigators is to be sought in the fact that they have studied different plants in which the types of mitosis may be very different, so that in one there may be a mass (quantitative) reduction, in another a numerical (pseudo) reduction, and in another a reducing (qualitative) reduction.

C. J. C.

**Häcker, Dr. Valentin.** Praxis und Theorie der Zellen und Befruchtungslehre. Jena, Gustav Fischer. Pp. VIII+260. 137 text figures, 1899. 8 marks.

This book is designed as a practical guide in the study of cell structures and fertilization both in plants and animals. It differs from such books as O. Hertwig's *Zelle und Gewebe*, and E. B. Wilson's *The Cell in Development and Inheritance*, in that it gives practical directions for collecting, preparing, and studying the various forms. As might be expected from a zoölogist, the author has made the zoölogical side very dominant, only five of the forty objects given in the table of contents being botanical. There are sixteen exercises, each

one including from two to four objects. The general plan is to present first the material and methods, then directions for study, and finally an historical résumé of the subject. The work furnishes an excellent introduction to the subject of cytology.

C. J. C.

**Stone, G. E.** Flora of Lake Quinsigamond. Mass. Agricultural College, July 1, 1899.

The list is especially rich in algæ. It is unfortunate that such lists are so rare, for it would be interesting to compare such complete aquatic floras of different regions. Permanent preparations were made of most of the filamentous algæ given in the list. The writer used for this purpose a mixture of one part glycerine, two parts water, and three parts alcohol. The algæ to be mounted were placed in this fluid, and the water and alcohol were allowed to evaporate. The mounts were then sealed in the usual manner.

C. J. C.

### RECENT LITERATURE.

**Butschli, O.** Untersuchungen über Structuren, with an atlas of 26 plates of microphotographs. Leipzig, 1898.

**Davis, B. M.** The Spore Mother Cell of *Anthoceros*. Bot. Gaz. 28: 89-109, pl. 9-10, 1899.

**Dixon, H. H.** Self-Parasitism of *Cuscuta reflexa*. Proc. Roy. Irish Acad. 3rd Ser. 5: 219-220, 1899.

**Fullmer, E. L.** The Development of the Microsporangia and Microspores of *Hemerocallis fulva*. Bot. Gaz. 28: 81-88, pl. 7-8, 1899.

**Hörmann, G.** Die Continuität der Atomverketzung, ein Structurprincip der lebendigen Substanz. Pp. 118, with 32 text figures. Jena, 1899.

**Klebs, G.** Zur Physiologie der Fortpflanzung einiger Pilze. II. *Saprolegnia mixta*. De Bary. Jahrb. f. wiss. Bot. 33: 513-593, 1899.

**Schenck, F.** Physiologische Charakteristik der Zelle. Pp. 123. Würzburg (A. Stuber), 1899.

**Shütt, F.** Centrifugales Dickenwachsthum der Membran und Extramembranöses Plasma. Jahrb. f. wiss. Bot. 33: 594-690, pl. 6-8, 1899.

**Zehnder, Ludwig.** Die Entstehung des Lebens. Erster Theil: Moneren, Zellen, Protisten. Freiburg i. Br., pp. 256, 1899.

## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
Agnes M. Claypole, Sage College,  
Ithaca, N. Y.

### CURRENT LITERATURE.

**Heidenhein, Martin.** Beiträge zur Aufklärung des wahren Wesen der faserförmigen Differenzirungen. Anat. Anz. 16: 97-131, 15 fig. in text, 1899.

The author has taken up work in a line considered by Benda (Weitere Mittheilungen über die Mitochondria. Verhdlg. der Phys. Geselsch. zu Berlin, 1898-99). That author states his conviction that the protoplasmic continuations of cilia on ciliated cells pass downward, and eventually past the nucleus, into the lower part of the cell, undergoing in their course actual confluence, so that fewer fibers exist when passing the nucleus than at the free end of the cell. Heidenhein found a most beautiful demonstration of the same condition in the epithelium of the intestinal tract of the frog; but it was found to be visible only when the cells were cut at a given plane. This epithelium has of course no cilia,



but is so closely similar to a ciliated epithelium that it shows similar structure. The author worked out for these cells a schematic form which he calls a four-sided column, with so large a nucleus as to nearly fill the cell. Plasma fibrils begin at the free end of the cell and pass to the opposite end, not, however, surrounding the nucleus entirely, but passing on three of the four sides, and crowding the nucleus slightly closer to the wall on the fourth side where the fibers are not present. These fibers were clearly seen to divide dichotomously, passing from the base outward, and form a cone or pencil incomplete on the one side. Ciliated cells from the liver passages of *Helix* were fixed in sublimate and stained in iron hæmatoxylin. These cells are either high cylindrical or flat in shape, but only the first kind were used in these studies. In this kind, many of the cells had entirely lost the general body substance, so that the fibrous part was naturally isolated. The basal bodies are very clearly developed, and a surface view of such cells shows them as spots, sometimes regularly and sometimes irregularly placed, and occasionally several are connected to form a continuous line. The author considers the fibers to form a beautiful example of Englemann's fibrous spindles.

A surface view of the disc, though not satisfactorily obtained in actual section, is well seen in optical section, and gives an appearance corresponding exactly to Cohnheim's areas in a cross-section of striated muscle. This observation brings the author to a general consideration of the transition between histological and molecular structures. He states that it is merely one of degree, not at all one of kind, and continues with a clearly drawn illustration from the structure of muscle. He shows that there is great difficulty in defining and designating a muscle fibril. Authors disagree as to what is the ultimate unit, and even the application of very high magnifications will not definitely solve the question, beyond the structure first recognized under moderately high powers. All resolution tends to simply show finer and finer subdivision of a similar nature, but no new or definite unit. In short, the only unit that can be reached beyond is the ultimate contractile unit, and this the microscope cannot discover. But the whole process is one of repeated subdivisions of Cohnheim's areas into smaller and smaller parts; there is no intermediate unit. Englemann's contractile units, the "Inotagmen," are these last molecules, and series of these arranged in rows form progressively larger and larger masses, until finally the range of microscopic visibility is reached, hence the structure seen but represents a massing of "Inotagmen" in strands. A single primary row of these units is assumed to be capable of assimilation, growth, and division; in this way masses arise that vary much in size. In other words, as the author puts it, the genetic significance of Cohnheim's areas lies in that there have arisen in the same field histological fibrils of higher or lower "orders," from the same original mother fibril or series of "Inotagmen." Again, he defines a muscle fibril as being what in each special case, with the given optical staining or other technical aids available, can be isolated from the metamicroscopic, fibrous structure of muscle as a visible fibrous unit. The histological unit in muscle tissue is simply determined by its visibility; there is practically no structural change from the first series of "Inotagmen" to the full-sized fiber.

Returning to Englemann's fibrous spindle in the ciliated cell, the author states that the only visible difference between what would be seen in a cross-section of this pseudo-cone of fibrils, and one of Cohnheim's areas, would lie in the greater uniformity of the former and its definite number of fibrils. If the section should pass above that part of the intracellular fibers where dichotomous division has occurred, the resemblance to muscle would be still stronger, as there would be groups of fibrils representing divisions of the respective original fibers. If it were possible to have a set of serial sections of this fibrous pseudo-cone from the ciliated cell, it would show a complete analogy to the gradual development of the cross-section of muscle, from the elementary fibril to Cohnheim's areas.

Taking another set of cells far removed from those already considered, for the sake of applying the explanation more widely, the author considers next the plasma-structures of white blood corpuscles. Shortly, the difference in their structure lies in that the "Inotagmen" have been arranged, not in parallel rows, but radiating from a center. We can consider that the spherical body of the leucocyte is composed of many sectors, each one of which corresponds to one of Englemann's ciliary structures. The point of each fibrous disc lies in the centrosome, or is in relation with it, but the fibers are infinitely finer than in muscle or epithelium. Further delicacy of structure in other cases, as the red blood corpuscle, will carry the fibrils below the limit of microscopic visibility, but they as certainly exist as when clearly seen in the largest cell. This idea is continued to include the much discussed polar radiation in mitosis. These Heidenhein considers to be always present, but in many cases so small as to be below the limit of microscopic vision. The cause for variation in size of fibrils is physiological, and the author leaves it undiscussed. In every way the paper is most interesting and suggestive.

A. M. C.

**Tsujitani, J.** Ueber die Reincultur der Amœben. *Centrbl. f. Bact.* **26**: 666-670, 1898. (*Abst. in Zeit. f. wiss. Zoöl.* **16**: 65-67, 1899.)

The author used three forms of amœbæ for these experiments, a kind of *amœba lobosa* obtained from hay infusion, a second kind from dust, and a third

from soil. They all grew in nutritive media at the room temperature, but body heat was more favorable. They liquify gelatin. Since these amœbæ sought and ate different kinds of bacteria, the author endeavored to separate them from resistant forms of bacteria, and to preserve with them non-resistant forms. The cholera bacillus was used for this purpose. A mixture of finely cut straw (30g.), *Gigartina prolifera* (10g.), and water (1000g.), was boiled for an hour in a steam sterilizer and filtered through clean cloth. After the addition of 1 to 1.5 per cent. of agar, the mixture was made alkaline with soda solution in the proportion of 1 to 100 parts. This mixture is again boiled for thirty to forty minutes, distributed, and sterilized. Finally, the condensation water of the nutritive medium is inoculated with some of the material containing amœbæ. After several days amœbæ and bacteria develop on the surface of the nutritive medium. Another mixture used was an alkaline medium of 1 to 1.5 per cent. agar in ordinary bouillon (20g.), and (80g.) of water, filtered, sterilized, and hardened on a slant. Later this is inoculated by a stroke with cholera bacillus, and in the condensation

water with amœbæ. The bacilli develop along the stroke in a line which is gradually eaten from below by amœbæ. This process can be observed under the microscope in a petri dish. Close to the border line between the eaten and not-eaten parts of the line of cholera growth, most of the amœbæ are seen. Other bacteria inoculated with the amœbæ are, as a rule, not developed, but if others are found a fresh culture is made. To prevent the growth of the undesirable forms over the surface of the culture, it was found efficacious to keep the tubes in the incubator for the first day, and after that at room temperature. To obtain pure cultures of amœbæ, such mixed growths are treated with acid and alkalies. This kills the cholera bacilli while the amœbæ withdraw into cysts. If a sterilized silk thread dipped into this mixture of amœbæ and cholera bacilli, is dried in a sulphuric acid drier, amœbæ alone remain living. These threads can then be used to make pure cultures. By inoculation from the cyst on gelatin or agar, the vegetative form is obtained, but no increase in numbers; this occurs alone in the presence of living or dead bacteria. Cover-glass preparations are made by mixing a small amount of culture with a platinum loopful of concentrated solution of acidified chinin. After spreading and drying in the air, it is treated with alcohol ether mixture, dried, and stained with methylen blue.

A. M. C.

**Determann.** Klinische Untersuchungen ueber Blutplättchen. Deutsch. Arch. f. Klin. Med. 61: 365-411, 2 pls, 1898, (Abst. in Zeit. f. wiss. Zoöl. 16: 86-88, 1899.)

In order to recognize and distinguish the separate blood plates, as well as to determine their numerical relations to the blood corpuscles present, the two conditions of massing and easy fragmentation must be considered. A simple addition of preservatives is not sufficient, as this causes a massing of the plates and change in their form. The desired result was obtained by pricking the finger and putting a drop of fixative on the spot. The blood first appearing is thoroughly mixed on a cover-glass with the preservative. Further dilution, if necessary, is made on the cover. From such a preparation the number and numerical relations can be easily determined, if care is taken to count the plates in the whole thickness of a preparation. For a preserving and diluting liquid the author used chiefly a .9 per cent. solution of ordinary salt, to every ten cc. of which had been added a drop of concentrated aqueous, well filtered methyl violet. A mixture of one per cent. sodium chloride with five per cent. bichromate of potash was also very successful. Of many other fluids the author preferred the following: Distilled water 160 g., glycerine 30, sodium chloride 1.0, sodium sulphate 8, methyl violet, .025 (according to Marchner). This was particularly good since it stains leucocytes and blood plates slightly, but enough to render their recognition easy. The plates vary in form from rods to round or oval discs, but after a short time they lose their shape, swell, and finally disintegrate.

For dried preparations the best stain was found to be a concentrated solution of methylin violet. Of chemical reagents used, aqueous solutions were found to act in the same time on both the plates and the corpuscles, taking hæmaglobin out of the latter. Corpuscles remain morphologically complete for some time, but the plates swell three or four fold, become globular, and finally break up in five to ten minutes. Weak acetic acid preserves the plates as long as the red corpuscles, and the leucocytes last longer than either.



To obtain the blood for determining the numerical relation of plates, the author used small blown glass tubes sealed at both ends; breaking off an end, the tube is filled from the fresh blood mass and the blood is received into a nearly airless tube. Instead of sealing the tube with heat, sealing wax was used, which was in every way satisfactory, and avoided the injury found to follow the heat required to fuse the glass. Blood so prepared was scarcely coagulated after twenty-four hours, but remained thin and a bright red.

A. M. C.

**Müller, Fr.** Die morphologischen Veränderungen der Blutkörperchen und des Fibrins bei der vitalen extravasculären Gerinnung. Beitr. z. Path. Anat. u. z. allgem. Path. 23: 498-528, 1 pl., 1898. (Abst. in Zeit. f. wiss. Zool. 16: 90-92, 1899.)

The author used for his studies the living blood clots formed by extravasation in the front chamber of the eye of the rabbit. By puncturing the cornea with a sterile, blunt-angled, sharpened

canula, an extravasation is obtained. By operating quickly and removing only part of the contents, the escape of the humor from the chamber can be prevented, and a repetition of the operation in the same eye is rendered possible. The liquid in the canula is put on a sterile Hollundermark cover, sealed with vaseline on a hollow ground slide, and examined under 800 or 1000 magnification. For fixation the covers were either heated on a copper plate, or dried in the air and treated with 10 per cent. formalin-alcohol (1 pt. 40 per cent. formaldehyde and 9 pts. of absolute alcohol). Concentrated sublimate solution, followed by iron hæmatoxylin stain, gave excellent results. Good results, but less certain, were obtained by treating with a four per cent. formalin solution, followed by alcohol of increasing concentrations. The liquids are allowed to flow drop by drop on the cover; a strong stream must be avoided. These are stained for half an hour in Delafield's hæmatoxylin, and for the same time in picro-fuchsin. Heidenhain's hæmatoxylin counter-stained with eosin and eosin methylen blue and triacid were used more rarely.

In fresh preparations amœboid movement could be seen in the leucocytes either with or without the use of neutral red. During coagulation the leucocytes can be seen to send out thick or thin thread-like processes, on which reddish granules were sometimes observed. As in the ordinary blood clot, the substance, after the destruction of the cell elements, appears as a homogenous membrane. The processes from the corpuscles soon become invisible. Shortly after the removal of the liquid from the eye, on the addition of neutral red, a reddish network of threads can be seen forming, enclosing the blood plates and corpuscles. Some fibers are especially broad, wavy, and homogenous, and these, after fixation with heat, remain only partially differentiated.

Similar results were obtained by raising a blood mass in the subcutaneous tissues on the back of a guinea pig. Six to eight Hollundermark covers are placed in the wound and left for three hours. On removal they are fastened together by a firm clot and are treated in a four per cent. formol and Müller-sublimate solution. These show the same network structures. The author concludes that these processes show fiber formation not typical of true fibrin.

A. M. C.



## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review  
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**Pfuhl, E.** Untersuchungen über die Entwicklungsfähigkeit der Typhusbacillen auf gekochten Kartoffeln bei gleichzeitigem Vorhandensein von Colibacillin und Bakterien der Gartenerde. *Centrblt. f. Bakt.* **26:** 49-51, 1899.

The colon bacilli used in this investigation were isolated from the fæces of three different individuals, and all were shown to possess no real motion in the hanging drop examination. The

Eberth bacilli, on the other hand, were all actively motile. Mixed cultures of the colon and Eberth bacilli were made in the following manner on potato. First a small amount from an agar culture of the Eberth bacillus was spread over the cut surface of a cooked potato, and the surface thus covered was then smeared with a colon culture. The mixed culture was placed in a moist chamber and kept in the incubator for seventeen hours. At the end of this time an examination was made of the upper layers of the excellent growth which had developed, but only colon bacilli were found. From the border of the growth material was now taken, the platinum loop being forced into the substance of the potato, and in the hanging drop made from this the Eberth bacilli were found, though few in number. After removing all apparent growth from the surface of the potato, hanging drops were made from the surface thus laid bare, and Eberth bacilli were shown in greater numbers, showing that the germ had penetrated the substance of the potato. Like results were obtained in all of the three Eberth and three colon cultures which were received from different sources. In one instance the cultures were examined for three successive days, and the Eberth bacillus was present each time.

Cultures were also made by streaking potatoes first with the Eberth bacillus and then with garden earth. The result was the same here except that it was necessary to isolate the Eberth bacillus by the plate method. From these investigations it is shown that the Eberth bacillus can grow on cooked potato in the presence of colon bacilli and of bacteria of various kinds found in garden soil, and that they penetrate the substance of the potato.

H. H. W.

**Dinwiddie, R. R.** The Relative Virulence for the Domestic Animals of Human and Bovine Tubercle. *Ark. Agri. Exp. Stat. Bull.* No. 57. June, 1899.

The results obtained by the investigation are briefly summed up as follows :  
**CATTLE**—*Feeding tests with tubercular sputa of man.*—Of four calves sub-

jected to this test all failed to give a tuberculin reaction, and, according to the post mortem examination, all remained absolutely uninfected.

*Inoculation of sputum and bovine tubercular material.*—Intra-tracheal inoculation was tested on two calves. The sputum-injected calf alone responded to the tuberculin test. The changes found on post mortem were, in both cases, slightly roughened patches on the pleura, without visible evidence of any tuber-

cular process; in the sputum test no local lesion, in the other a number of abscesses in which tubercle bacilli were found.

Intra-peritoneal inoculation; four calves in each series. Of the sputum series, two gave doubtful and two negative reactions to tuberculin; of the bovine series, two positive and two negative. The pathological changes in the sputum lot were: in one no lesions, in another a few roughened patches and seed-like granules on the omentum, with slight fringe formation on various parts of the abdominal serosa, a few whitish spots on surface of liver. The fourth was not examined post mortem.

The bovine series showed: in one no lesions, in two changes similar to the above but somewhat more extensive; in the fourth a very extensive development of grape-like growths, containing tubercles all over the peritoneum, a few of same on thoracic surface of diaphragm, tuberculosis of some of the abdominal lymph glands.

*Comparative tests with sputum and bovine cultures.*—Intra-tracheal inoculation on two calves. The sputum-tested animal gave no reaction to tuberculin. The post mortem findings were slight pleuritic adhesions, fringe development on costal pleura, a few lymphoid nodules of the size of millet seed histologically showing no relation to tubercles in lungs. Bovine culture: locally a number of abscesses external to trachea, and fungus-like ulcerating growths on its mucous surfaces, all containing tubercle bacilli; lower cervical lymph nodes tubercular.

Intra-peritoneal inoculation: two calves, sputum culture. Reaction to tuberculin. On omentum are several patches studded with millet-seed-like calcareous granules, and one small bean-sized polypoid growth; a few mesenteric glands, tubercular. Bovine culture: fatal result in six weeks. Acute tubercular disease involving all the abdominal and thoracic organs; lesions in the form of miliary tubercles, large yellow nodules and fibrous grape-like growths on serous surfaces; tubercle bacilli very abundant.

Inoculation in lung: two calves, sputum culture. No tuberculin reaction; no post mortem. Bovine culture: tuberculin reaction; abscess with surrounding consolidation and tubercles in lung; numerous tubercles in pleura; fringe formation; tuberculous bronchial glands.

Feeding: two calves; sputum culture. No reaction to tuberculin; no post mortem. Bovine culture: reaction to tuberculin; mesenteric glands tubercular, many yellow spots on liver.

*Pigs.*—Feeding sputa to six pigs resulted in infection in two, the disease being confined to the submaxillary and mesenteric glands.

*Intra-peritoneal inoculation of sputa and bovine tubercular material.*—Four pigs; sputum lot. One died from other disease before effects could be produced. The other shows a local lesion, a general tuberculosis of abdominal serosa, tuberculous abdominal and thoracic lymph glands, and embolic tubercles in lungs. Bovine lot: both presented essentially the same condition as the last described, in the abdominal cavity, but no extension to thorax.

*Intra-peritoneal inoculation of cultures.*—Two pigs; sputum culture. No lesions. Bovine culture: in one generalized, in the other localized in submaxillary and mesenteric glands.

**SHEEP**—*Intra-peritoneal inoculation of cultures*.—Two sheep; sputum culture. Patch of seed-like granules on omentum. Bovine culture: generalized disease, ending fatally.

**CHICKENS**—*Feeding sputa*.—Six chickens, all unaffected.

*Intra-abdominal inoculation of sputum and bovine cultures*.—Ten chickens. Both lots were unaffected.

A comparison of the virulence for cattle of human and bovine tubercular material seems to show that the bovine material is more virulent. For pigs there seems to be no difference in virulence, both being capable of producing infection. Sheep are highly susceptible to bovine tubercle bacilli. The results in regard to human tubercle bacilli were not sufficiently worked out to arrive at a definite conclusion. Chickens were very insusceptible to both. It also seems that there can be no doubt that cattle are susceptible to infection from human tubercular material, and that the disease is similar to the naturally acquired tuberculosis of cattle, though the lesions are usually much less extensive. All comparative tests, with the exception of that of Chauveau, show a greater susceptibility of cattle for bovine than for human tubercular material.

H. H. W.

**Beco, L.** Note sur la valeur de agglutination par le sérum antityphique experimental comme moyen de diagnostic entre le bacille d'Eberth et les races coliformes. Centrblt. f. Bakt. 26: 136-139, 1899.

The author has tested the action of anti-typhoid serum and formalin on cultures of the Eberth and colon bacilli obtained from various sources,

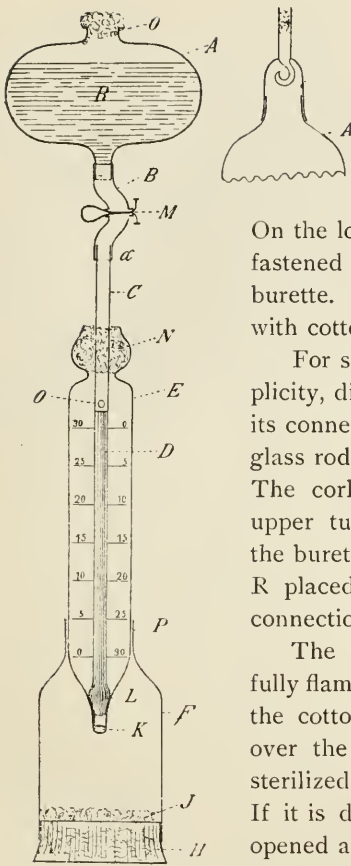
and has drawn the following conclusions from his observations: Agglutination caused by an experimental antityphic serum is a valuable and practical means for the differentiation of the Eberth-Gaffky bacillus, provided that the serum used is very active. The test should only be considered positive when agglutination takes place with a dilution considerably less than the dilution with which the most sensitive of the colon group responds to the reaction. Antityphic serum is endowed with a very variable power of agglutination with respect to the colon bacillus. In some cultures apparently morphologically and biologically identical there is no reaction, in others it is considerable. Formalin does not agglutinate certain well established typhoid cultures. On the other hand, it frequently causes the agglutination of the colon bacillus and other bacteria often met with in the stools and in water. The conclusions drawn from these facts is that the agglutinating property of formalin is of interest theoretically rather than practically.

H. H. W.

**Epstein, S.** Apparat zum sterilen Abfüllen von Flüssigkeiten. Centrblt. f. Bakt. 26: 1te. abt., 34-35, 1899.

The apparatus consists of a vessel, R, for the sterile fluid, a graduated burette, E, which fits into a bell-jar, F, closed

below with a cork upon which is placed a thin layer of wool. The glass vessel R is made broad so as to do away with as much height as possible, and is open both above and below. The upper opening of R is wide and closed with cotton. To prevent evaporation a ground-glass cap with a spiral glass rod which is closed above with cotton, was used (Fig. 2). The lower opening of R is drawn out and serves as a means for connecting the glass vessel R with the burette E.



The burette E, which is graduated, is closed at L by a glass rod, O, which is conical at the lower end and serves as a stopper to the burette at L. Below is an opening, K, which acts as an outflow. The glass rod consists of two parts, the upper, C, hollow with an opening at O, the lower, D, of solid glass.

On the lower end of the burette at P is a bell-jar either fastened by a rubber band or soldered directly to the burette. The upper part of the burette is closed at N with cotton.

For sterilizing the apparatus it is, for the sake of simplicity, divided into two parts. The upper vessel R with its connection B is taken and closed at  $\alpha$  with a small glass rod, and together with the pinchcock M is sterilized. The cork below is removed with the burette and the upper tube C covered with cotton. After sterilization the burette is held fast by a suitable stand, and the vessel R placed in a corresponding ring so that the rubber connection B can be easily drawn over the tube C.

The lower end of the rubber connection is now carefully flamed with a Bunsen burner, the glass rod removed, the cotton stopper burned, and the rubber tube drawn over the tube C. The bell-jar is then closed with a sterilized cork upon which has been placed a little cotton. If it is desired to use the apparatus the pinchcock M is opened and the burette filled up to the desired mark. By lifting the glass rod C the conical end L is raised and the

burette emptied. If the cork stopper has to be taken out, it is advisable to moisten the cotton layer with two or three drops of a 40 per cent. formaldehyde solution.

H. H. W.

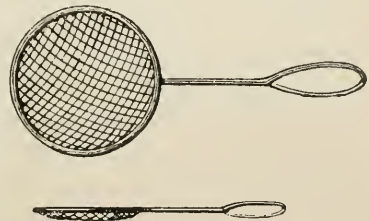
**Korn, Otto.** Eine einfache Vorrichtung zum Erhitzen der Farbstofflösung bei der Tuberkelbacillen-färbung. Centrbl. f. Bakt. ite Abt. 25: 422-423, 1899.

The writer prefers to stain cover-glass preparations by floating them, bacillus side down, in a watch glass of hot staining fluid, instead of heating the

stain on the cover-glass, as this process drives the water out so rapidly that crystals are likely to be formed.

The figure shows a convenient device for heating the watch glass. A piece of coarse wire is bent to form a handle at one end, and the rest of it is made into a circle measuring from five to seven cm. Over this a slightly concave piece of wire netting is placed and the edges bent around to fasten it to the circular frame. The watch glass is easily handled with this, and the wire netting keeps the flame from cracking the glass.

E. M. BRACE.





**Smith, Theobald.** Some Devices for the Cultivation of Anaërobic Bacteria without the Use of Inert Gases. Jour. Bost. Soc. Med. Sc. 3: 340-343, 1899.

The development of anaërobes in fermentation tubes suggested their cultivation in culture flasks. The apparatus described (Fig. 1) is simple and easily manipulated, and was used more particularly for the cultivation on a large scale of the tetanus bacillus for the production of a tetanus antitoxine. The method proved more satisfactory than the removal of air by aspiration, with the substitution of hydrogen.

There are two bulbs, A and B, connected by a heavy rubber tube, *c*, and with a clamp, D, to regulate the communication between them. The bouillon ordinarily fills A and the space below the dotted line in B, but during sterilization it is forced over into B. It is inoculated through the cotton-plugged opening E. The growth will extend around to A within twenty-four hours. A may be made with

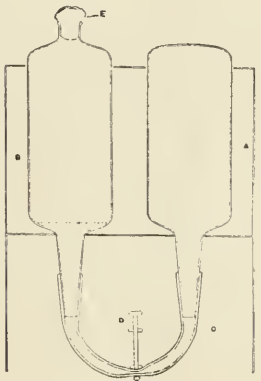


Fig. 1.

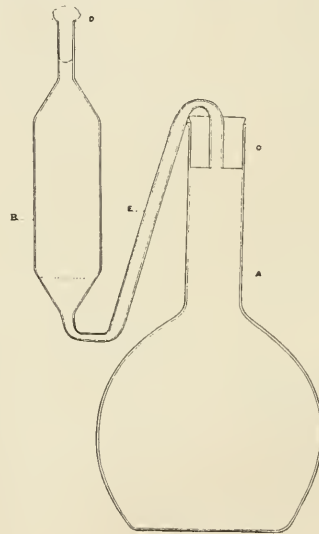


Fig. 2.

an opening at the top for convenience in filling and cleaning, but in that case it must have a rubber stopper wired down to exclude the air. The tin rack F is convenient for holding the apparatus.

Fig. 2 shows a variation of the apparatus, in which a liter flask, A, and a bent 100cc. pipette, B, are used. The upper part of the pipette is shortened and plugged with cotton, while the lower part is bent and fitted to the flask with a rubber stopper. The bouillon is inoculated through the opening at D, and the growth reaches the flask in twenty-four to thirty-six hours. The second form cannot be autoclaved when filled, as some of the fluid will be thrown out. To obviate this, the flask is only partly filled and the extra bouillon required is autoclaved with it in an ordinary flask. There is no difficulty if the Arnold sterilizer is used.

E. M. BRACE.

**Darling, E. A.** Observations on the Sterilization of Catgut. Jour. Bost. Soc. Med. Sc. 3: 269-273, 1899.

**Hirst.** A Method of Preparing Catgut. Berliner Klin. Wochensh. Nos. 26-29, 1898.

From experiments made by Darling upon different ways of sterilizing catgut, to determine the effectiveness of the methods, with the changes produced in the strength of the threads, it was found that catgut can be sterilized by dry heat with but slight loss of tensile strength, that dry paraform gas is of doubtful value for sterilization, and that methods which involve soaking in antiseptic solutions tend to weaken it. The weakening effects of solutions are tabulated. The brittleness which results from exposure to dry heat may be prevented by wrapping the separate strands in several layers of paraffin paper. Material heated to 140°C. for three hours will be thoroughly sterilized.

Hirst finds that catgut is not always perfectly sterile even when it has been boiled in alcohol at 240°F. and gives a method for absolute sterilization. Soak the catgut in benzine twelve hours to remove the fat, dry on blotting paper, soak in sterile water to make it absorptive, and immerse in a five per cent. solution of formalin for about fourteen hours. Wash out the excess of formalin, stretch on a form, leave in a warm room four or five days until perfectly dry. Then wind on a large wooden spool so that the coils will not cross, and put it in a ten per cent. solution of glycerine in absolute alcohol. It is then sterilized for forty-five minutes or an hour in a metal cylinder with a tight screw cap, placed in an autoclave sterilizer and kept at 240°F. Catgut treated in this way will last seventeen days or more, according to where it is used, and micro-organisms will not grow on it after the treatment with formalin.

E. M. BRACE.

**Pagenstecher.** Celluloid Thread. Deutsche Med. Wochensh. April 6, 1899.

Thread is boiled for one-half hour in a one per cent. solution of soda, washed in boiling water, and dried between sterile compresses. It is then soaked in a solution of celluloid and passed through the soda solution a second time, after which it is sterilized by steam. It may be kept ready for use in an alcoholic solution of corrosive sublimate, or dry. These threads may be used instead of silk for sutures and ligatures, and in many cases they are preferable to catgut.

E. M. BRACE.

**De Klecki, C.** Contribution à la pathogénie de l'appendicite. Ann. de L'Inst. Pasteur. 13: 480-499, 1899.

From experiments made on the rabbit it was shown that it was not necessary to occlude the appendix in order to exalt the virulence of the colon bacillus. Stagnation of the contents of the appendix plays an important role in the pathogenesis of appendicitis. Stagnation, except of the gaseous contents, is produced in the advanced stage of every case of appendicitis, with or without occlusion of the appendix, from paralysis of the wall of the intestine. The occlusion of the appendix, then, does not play the predominating role in the pathogenesis of all appendicitis.

It was also shown by these observations that a suppurative appendicitis with a predominance of colon bacilli in the pus from the appendix, may arise without exaltation of the virulence of this germ, by simple alteration of the nutrition of the walls of the appendix. A lessened resistance of the middle wall of the appendix plays an important role in the pathogenesis of appendicitis. Acute appendicitis always takes place in an organ of which the wall is altered by chronic

pathological processes. It is very probable that these processes induce the lessened resistance of the appendicular wall. The role of this agent should be more and more evident in all infections. In non-specific appendicitis, its importance is so much the greater since infection is always secondary in this affection.

H. H. W.

**Pearce, R. M.** Scarlet Fever, its Bacteriology, and Gross and Minute Anatomy. Med. and Surg. Rept. Bost. City Hosp., 1899.

From bacteriological investigations no light was thrown on the etiology of scarlet fever. The microorganisms

found, named in the order of their frequency, are the streptococcus pyogenes, the staphylococcus pyogenes aureus, and the pneumococcus. The streptococcus is found to be the most frequent cause of general infection, and of infection of the mucous membranes of the nose and throat. There is little if any evidence that any of these organisms have anything to do with the etiology of scarlet fever. Their presence is in all probability due to secondary infection. Infections of the middle ear, antra of Highmore, and of the sphenoidal sinuses are of much importance since they are present as complications in a large percentage of the cases of scarlet fever, and if in these cases death does not result, these secondary infections may produce chronic inflammatory sequelæ.

H. H. W.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Harvard Medical School, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

### CURRENT LITERATURE.

**Bezancon et Labbé.** The Ganglion Lymphatique. Normal Anatomie et Physiologie. La Presse Medicale. Feb. 15, 1899.

The greater part of this article is devoted to the histology of the lymph node. The description is briefly as

follows: The capsule is formed of bundles of fibrous tissue in which are elastic fibers, and in some animals, as the ox, horse, and mouse, smooth muscle fibers. It contains few blood vessels, but many lymphatics. The capsule, forms a complete envelope, and at the hilum is reflected on to the vessels, passing with them into the organ. The arteries, on reaching the lymph nodules (so-called follicles) in the interior of the node, divide into a close network of capillaries which radiate through the nodule like the spokes of a wheel ("*disposé en rayons de roue*").

The tissue proper is composed of a delicate reticulum in which lie the essential cells of the node. The writers do not attempt to decide whether the reticulum is made up of anastomosing star-shaped cells (explanation of Kolliker, His, and Frey) or of anastomosing fibrils lined by endothelial cells (explanation of Ranvier).

The essential cells are the lymphocytes, cells with round nuclei rich in chromatin, and surrounded by a slight amount of protoplasm, which when more

fully developed the writers consider to be identical with the mononuclear leucocyte of Ehrlich.

In the follicles, in addition to the typical lymphocytes, is found an almost equal number of cells closely resembling them but with a larger nucleus, in which the chromatin is not so compactly arranged. A few small mononuclear cells and a few eosinophiles are also found.

Some follicles are uniformly composed of lymphocytes in which no evidence of karyokinesis is seen. Other follicles have a peripheral zone composed of lymphocytes and a "clear center" (germinative center) made up of what the writers call mononuclear leucocytes with numerous karyokinetic figures. There is no sharp line between these two kinds of cells. The protoplasm of some of the larger cells in these centers contain fragments of chromatin which would indicate that they are phagocytic in character.

In the subcapsular sinuses are found lymphocytes, mononuclear leucocytes, small and large eosinophiles, and cells of the reticulum, which are sometimes seen to have engulfed red blood corpuscles and lymphocytes. In the cavernous sinuses practically the same cells, with occasionally mast cells, are found, though the phagocytic cells are not so common.

Never in the normal lymph node (guinea-pig and rabbit), say the writers, are polynuclear leucocytes found. The lymph nodes used for study were those of the guinea-pig and rabbit, and differed little according to location. The bronchial nodes had a greater vascularity, and the mesenteric showed a few slight differences in their reticulum.

In the cat the follicles are very abundant. In the dog there is an abundance of connective tissue.

In man the capsule is less developed and cannot be seen penetrating the node. There are very few eosinophiles. The "germinating centers" of the follicles are much more prominent in the child than in the adult. In the latter, connective tissue proliferation is prominent and there is little evidence of karyokinesis. In old age a true atrophy takes place.

The function of the lymph node is to form the lymphocytes of the blood, and in the rabbit and guinea-pig probably the eosinophiles. This function is increased in infection as is also the production of phagocytic cells which engulf not only bacteria, but also dead cells and other foreign debris. R. M. P.

**Lochte.** Zur Kenntniss der epithelioiden Umwandlung der Thymus. *Centrib. f. Allg. Path. u. Path. Anat.* 10: No. 21, 1899.

Twenty cases of persistent thymus in the adult were studied. In one case, a male aged twenty-one who died of

septic purpura, the gland was almost entirely composed of large epithelioid cells with broad protoplasmic borders and large oval or round nuclei. The cells lay in an alveolar connective tissue. These cells probably arise from the cells of the reticulum.

Occasionally these cells showed unmistakable transformation from connective tissue cells. Some had two, three, or more nuclei. Small collections of lymphoid cells with colloid Hassall's bodies were found lying between the epithelioid cells.



In a case of acute leukæmia the same epithelioid cells, thickly crowded and often arranged in strands and bands, were found.

In order to determine whether any relation exists between these changes and acute blood diseases, seventy other glands were examined without finding similar transformations. Negative results were also obtained in cases of chronic leukæmia and pernicious anæmia.

A. M.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace, Biological Laboratory,  
University of Rochester, Rochester, N. Y.

Larionow, Dr. W. Ueber die musikalischen  
Centren des Gehirns. Archiv f. Physiol.  
76: 608-625, 2 text figs., 1899.

This is a study in localization of function contributed from the clinic of neuro-psychiatry of Prof. W. Bechteren, in St.

Petersburg. Trained dogs were used for the experiments. Portions were removed from the cortex of the temporal lobe and the hearing of the dogs was tested with sounds of different pitch and timbre. Perception of any sound would be indicated by turning the head or the eyes, motion of the ear, etc. The results show that there is a center for the perception of a tone scale corresponding to the tone scale of the cochlea. Extirpation of the second convolution of the temporal lobe destroyed the perception of tones between A<sup>1</sup> and e, of the third convolution, tones from e to c<sup>2</sup>, and of the posterior half of the fourth convolution (gyrus angularis), tones above c<sup>2</sup>. Removal of the entire cortex of the temporal lobe diminished the hearing for the opposite, and produced a slight effect on the corresponding, side, showing that most of the auditory fibers cross to the opposite ear. This was also shown in microscopical preparations made by Marchi's method. Hearing was not affected in control specimens having the frontal and parietal lobes destroyed.

Comparing the dog's brain with the human brain, the posterior third of the second convolution would correspond to the second convolution of the temporal lobe, the posterior third of the third convolution to the first convolution in man, and the posterior half of the fourth convolution to the posterior transverse convolution of the Island, in man. The part corresponding to the fourth convolution of the dog is concealed in man by the great development of the frontal and temporo-parietal gyri (anterior and posterior association centers of Flechsig), and forms the Island of Reil.

The word center of the dog is in an embryonic condition. It lies in the middle third of the third left gyrus, corresponding to the first gyrus of the temporal lobe in man.

Microscopical examination of sections prepared after Marchi's method showed the ending of the auditory tracts in the tone centers. Larionow agrees with Flechsig that the auditory tracts are double in the hemispheres.

Further observations for the human brain locate the center for note-reading

with the common reading center, for note-writing with the word-writing center, the song center a little back of Broca's motor center for speech, at the foot of the third left frontal gyrus, and the center for conception of musical ideas in the left frontal lobe near the anterior association center of Flechsig. The left tone center and the word center of Wernicke are distinct from each other and have separate fiber tracts.

The cause of tone deafness may lie in the ear, in its connection with the brain, or in the cells of the brain, and deafness for certain tones alone may exist.

E. M. B.

#### RECENT LITERATURE.

- Werigo, Prof. Br.** Zur Frage über die Beziehung zwischen Erregbarkeit und Leitungsfähigkeit des Nerven. *Archiv f. Physiol.* **76**: 552-608, 7 text figs., 1899.
- Beck, A.** Ueber künstlich hervorgerufene Farbenblindheit. **76**: 634-640, 1899.
- Zenneck, G.** Ueber die chemische Reizung nervenhaltiger und nervenloser (curarisirter) Skelettmuskeln. *Archiv f. Physiol.* **76**: 21-58, 21 text figs., 1899.
- Grober, Dr. Jul. A.** Ueber die Athmungsinnervation der Vögel. *Archiv f. Physiol.* **76**: 427-470, 8 text figs., 1899.
- Spina, Prof. A.** Ueber eine Methode, an gehirn-und rückenmarklosen Säugethieren zu experimentiren. *Archiv f. Physiol.* **76**: 219-222, 1899.
- Werigo, Prof. B.** Ueber die Reizung des Nerven mit dreiarmligen Elektroden. *Archiv f. Physiol.* **76**: 517-530, 6 text figs., 1899.
- Holl, M.** Ueber die Insel des Carnivorgehirnes. *Archiv f. Anat. u. Entwickl. H.* 3 and 4, 217-263, Tff. XI-XIII, 1899.
- Denker, Alfred.** Zur Anatomie des Gehörorgans der Säugethiere. *Archiv f. Anat. u. Entwickl. H.* 3 and 4, 207-214, Tff. X, 1899.
- Lubosch, Wilh.** Vergleichend-anatomische Untersuchungen über den Ursprung und die Phylogenese des N. accessorius Willisii. *Archiv f. Mikr. Anat.* **54**: 514-602, Tff. XXVII, 1899.

## NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA MCL. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

**Baumhauer, H.** Ueber sogenannte anomale Aetzfiguren an monoklinen Krystallen. *Zeit. f. Kryst.* **30**: 97, 1898.

The very important part played by the so-called etch figures in determining the structural symmetry of a crystal,

lends importance to this discussion of etch figures of unexpectedly low grade of symmetry. The mineral colemannite, which is here especially studied, occurs in well formed crystals of apparently definite monoclinic symmetry, and the optical tests confirm this, but the etch figures developed on (010) are of three distinct types, two of which are holohedral monoclinic, but the third of distinctly a lower grade. Similarly in the mineral diopside there are obtained two definitely monoclinic shapes, and a third anomalous shape of lower symmetry.

The suggestion is made and discussed that in both cases the anomalous figures have developed by a blending of the two different monoclinic figures, and it is pointed out that in general, the anomalous figures are not sharp, but somewhat curved, and frequently have prolongations which pass into the normal

figures; also that with the mineral apatite, the deeper, darker etch figures sometimes enclose smaller figures of the other type.

The conclusion appears to be that etch figures of unexpectedly low grade of symmetry, while undoubtedly due to structural peculiarities, are not to be hastily interpreted, and are not thoroughly understood phenomena analagous to optical anomalies.

A. J. M.

V. Kraatz-Koschlau, K. u. Wöhler, L. Die natürlichen Färbungen der Mineralien. Tschermak's Min. u. Petr. Mitt. 18: 304, 1899.

The view at one time held, that colors uniformly distributed but not properly belonging to a substance, were due to a mechanical inclusion of foreign coloring substance, so finely divided as to be beyond the range of our microscopes, has been abandoned for the theory of isomorphic replacement and a large series of colored solid solutions (feste Lösungen of Van't Hoff) have been artificially made.

The nature of the coloring material has been assumed by most to be usually organic, and in a few known instances inorganic. Weinschenk has lately, however, on the basis of an examination of smoky quartz, theorized that the coloring substances are in general inorganic, ascribing the color of smoky quartz, smoky topaz, and octahedrite to a sesquioxide of titanium, that of zircon to a sesquioxide of zirconium.

The authors have tested qualitatively and quantitatively a number of minerals from many localities, and find no support for the theory of Weinschenk, but on the contrary find that with a number of minerals, when heated in presence of oxygen, they obtained the smell of charred organic matter, phosphorescence and evolution of  $\text{CO}_2$  while the color lasted, but on the decolorized matter these tests were negative. According to their results, the following classification results:

1st. *Coloring Material Entirely Organic.*—Fluorite, apatite, barite, celestite, anhydrite, calcite, halite, smoky topaz, zircon, microcline, rubellite, topaz.

2d. *Coloring Material Partly Organic.*—Apatite of Canada, amethyst, topaz of Brazil, etc.

3d. *Coloring Matter Chiefly Inorganic.*—Ruby, sapphire, spinel, beryl, etc.

The percentages of carbon and hydrogen were determined quantitatively. An interesting series showing the relation of color to these percentages is that below:

		Per cent. C.	Per cent. H.
Violet	fluorite of Wolsendorff,	0.017	0.0038.
"	" " Schneeberg,	0.014	0.0038.
"	" " Weardale,	0.010	0.008.
Blue and green	" " Cumberland,	0.009	0.002.
Green	" " Beaiyolais,	0.0095	0.0025.
"	" " Hesselbach,	0.008	0.001.
Yellow	" " Dunham,	0.007	0.0025.
Colorless	" " Cumberland,	0.00	0.00.

A. J. M.

Tutton, A. E. Ein compensations Interferenz-dilatometer. Zeit. f. Kryst. 32: 529, 1899.

In order to determine the thermal expansion in plates of artificial crystals not usually thicker than 5 mm., Mr. Tutton has added to the Benoit Platinum-

Iridium type of Fizeau apparatus, in which the effective expansion is the difference between the expansion of the crystal and that of the screws of the tripod; an extra piece made of aluminum, the expansion of which is two and one half times that of the Pt Ir alloy for the same temperature change.

The proper thickness of aluminum to counteract the expansion of the known length of Pt Ir screw, is introduced, usually in the form of a cylinder resting on top of the crystal plate. The upper surface of the aluminum cylinder is polished. On heating, the screws expand, raising the glass wedge which rests upon them, the crystal and aluminum cylinder also expand in the same direction, and the effective change in the thickness of the film between glass and aluminum is due *only* to the expansion of the crystal.

An elaborate forty-page description of apparatus and its use is given.

A. J. M.

## NEWS AND NOTES.

ONE METHOD OF TEACHING PHYSIOLOGICAL BOTANY.—A little experiment was made during the spring term of 1899. It so happened that the Junior and Sophomore classes were to take botany together. Each class had had botany in the spring term of the Freshman year under the same instructor, using the same text-book, with the exception of one young lady in the Freshman class who had never studied the subject. MacDougal's "Plant Physiology" was used as a text-book and supplemented by twelve others as the case required. Lectures were occasionally given by the instructor which consumed only a part of the daily period. The rest of the periods of 55 minutes each were consumed in hearing, discussing, and taking notes on reports made by the members of the class on literature that had been previously assigned to each member. Nine periodicals devoted entirely or in part to botany and elementary texts on botany was the literature on which reports were required. There was one period of two hours per week devoted to making experiments, usually such as the student might select either from the leading text or some of the supplementary texts. In this way the students were interested from the beginning to the end of the term. Interest on the part of the student is perhaps the prime essential for successful teaching. Nearly all of the class, particularly the young men, seemed delighted to have an opportunity to report fully (and as freely as time would permit) what they had found in the literature assigned. The instructor would sometimes take a seat in the class and let the student have the floor completely for the time being. He would instruct without assuming that the students knew a good deal that they did not, which some instructors are quite liable to do.

When the end of the term came all of the class passed except two young women.

One of the interesting results was that among the young women, the one who had never before studied the subject passed highest.

This kind of work could not probably be so well done by the ordinary students of a Freshman class, nor could the scheme be so thoroughly carried out in large sections of classes.



From the nature of the subject but little microscopical work was either attempted or necessary. Experience teaches that the compound microscope should be brought into daily use late in a course in botany. E. E. BOGUE.  
Agricultural and Mechanical College, Stillwater, O. T.

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SUMMER WORK AT THE U. S. FISH COMMISSION LABORATORY, WOOD'S HOLL, MASS.—The hatching season at the U. S. Fish Commission Laboratory in Woods Holl usually ends about June 15, with the completion of the lobster work, and from this time until the hatching of cods' eggs is begun, about October 1st, the laboratory is practically given over to the investigators, who assemble there from various portions of the country, attracted by the opportunities afforded for the study of marine forms.

During the past summer, owing to the fact that the commission steamer *Fish Hawk* has been stationed at Woods Holl, special advantages for work in certain lines have been afforded. Frequent dredging trips have been made, not only in the neighborhood, but also in some cases to a considerable distance from Woods Holl. Opportunity has thus been given to determine the nature of the bottom fauna in various locations.

The schooner *Grampus* was also at the commission for a portion of the summer, and during that time made a number of trips to the waters bordering the Gulf Stream, in order to determine the boundaries of the region occupied by the tilefish (*Lopholatilus chamæleonticeps*). These fish, first found in 1879, became for a time extinct, so that from 1882 to 1892 no fish were taken, although careful search was made in their former habitat. After this disappearance of ten years, scattered specimens were again found, but it was not until August of last year that the fish were taken in abundance. At that time, as a result of several trips, over 3000 pounds of fish were caught. The expeditions this season have been equally successful, seeming to indicate that the tilefish have returned to their old location. The region in which they are at present found lies off the southern coast of Massachusetts and Rhode Island, in water ranging from 70 to 100 fathoms in depth.

Dr. H. C. Bumpus, the director of the scientific work of the commission, has been conducting experiments upon the rearing of young lobsters, with the aim of finding some method by which they may be kept alive in confinement for a considerable period. At present it is necessary to liberate the lobster fry almost as soon as hatched. The result of the work carried on this season seems to indicate that the lobster can be kept for a considerable time before being liberated, and thus the mortality of the young lobsters be greatly decreased. This will be of immense practical value in the effort which is being made to prevent the extinction of the lobster along our eastern coast.

In addition to this work, some thirty or more investigators have been carrying on various lines of research at the commission during the summer.

University of Nebraska.

FRANK E. WATSON.

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AMERICAN MICROSCOPICAL SOCIETY.—The twenty-first annual meeting was held in Columbus, Ohio, August 17, 18, and 19. Though not largely attended

it was still the largest meeting ever held in the region west of New York State, and was the occasion of great interest and enjoyment to those who were present. The sessions of the society were held in the beautiful new Biological Hall of the Ohio State University, and the spacious campus with its magnificent trees made the sojourn delightful despite the heat. The society was welcomed by President Thompson of the University, and in responding Dr. Krauss referred to the debt which the society already owed Ohio State University, in the long and devoted services of a former member of its faculty, Professor D. S. Kellicott. At the various sessions of the society the following papers were read and discussed:

"An Expedient in Difficult Resolution," by Dr. R. H. Ward, of Troy, N. Y.

"The Relation of Cancer to Defective Development," by Dr. M. A. Veeder, of Lyons, N. Y.

"Notes on Laboratory Technique," by Professor S. H. Gage, of Ithaca, N. Y.

"Recent Progress towards a Bibliography of Natural Science," by Professor Henry B. Ward, of Lincoln, Neb.

"The Reaction of Diabetic Blood to Some of the Aniline Dyes," by Dr. Vida A. Latham, of Chicago, Ill.

"Notices of Some Undescribed Infusoria from Louisiana," by Mr. J. C. Smith, of New Orleans, La.

"Modern Conceptions of the Structure and Classification of Diatoms," by Professor C. E. Bessey, of Lincoln, Neb.

"The Comparative Structure of the Soft Palate," by Mr. W. F. Mercer, of Ithaca, N. Y.

"A New Microscope Stand," by Dr. A. G. Field, of Des Moines, Iowa.

"The Eyes of Typhlomoge from the Artesian Well at San Marcos," by Professor Carl H. Eigenmann, of Bloomington, Ind.

"Methods Employed in the Study of the Chiasma of *Bufo vulgaris*," by Mr. B. D. Myers, of Ithaca, N. Y.

"Indexing, Cataloguing, and Arranging Microscopical Literature and Slides," by Dr. R. H. Ward, of Troy, N. Y.

Among other papers on the program which were read by title were the following:

"*Natogonia Ehrenbergii* Peitz," by Mr. J. C. Smith, of New Orleans, La.

"Milk as a Culture Medium," by Mr. Floyd R. Wright, of Ithaca, N. Y.

"Limnobiology and its Problems," by Professor Henry B. Ward, of Lincoln, Neb.

"A New Spencer One-twelfth Objective," by Mr. Henry R. Howland, of Buffalo, N. Y.

"An Apartment Incubator for Student Use," by Professor Veranus A. Moore, of Ithaca, N. Y.

"Notes on New Genera of Water Mites," by Professor Robert H. Wolcott, of Lincoln, Neb.

"Experiments in Antisepsis," by Dr. P. A. Fish, of Ithaca, N. Y.

"A Study of the Sun Animalcule," by Professor J. D. Hyatt, New Rochelle, N. Y.

"Some Essential Methods for Young Laboratory Instructors in Bacteriology," by Mr. Raymond C. Reed, of Ithaca, N. Y.

"The Plankton of Echo River, Mammoth Cave," by Dr. Charles A. Kofoid, of Urbana, Ill.

The annual address of the president, Dr. William C. Krauss, of Buffalo, N. Y., was given on Thursday evening, on the subject "Some of the Medico-legal Aspects of Diseased Cerebral Arteries." It impressed all as a strong effort in a comparatively new field. The afternoon of Friday was given to a symposium on the possibilities of microscopical work by teachers and private workers. The field of Animal Histology was treated by Professor S. H. Gage and Mr. B. D. Myers, of Ithaca, N. Y.; Bacteriology was treated by Prof. A. M. Bleile, of Columbus, O.; and Botany by Professor C. E. Bessey, of Lincoln, Neb. The various phases were very generally discussed, and the session proved of great interest to all present.

The report of the treasurer showed that the society had closed the preceding year practically even, with a total expenditure of about \$800, and that the Spencer-Tolles fund had been increased during the year by \$96, making it now nearly \$700. It is hoped that this fund may soon reach a point when the income may be devoted to the encouragement of research in some line connected with the microscope.

There was prepared for transmission to the Royal Society of London a memorial touching upon the matter of a bibliography of science. The society also decided, upon recommendation of the executive committee, to form sub-committees for the advancement of any special line of microscopical research in which conditions were especially favorable to an advance, and further to inaugurate this policy by the appointment of a limnological committee.

The following officers were elected for the year 1899-1900 :

President, Professor A. M. Bleile, Columbus, O.

Vice-presidents, Professor C. H. Eigenmann, Bloomington, Ind.; Dr. M. A. Veeder, Lyons, N. Y.

Treasurer, J. C. Smith, New Orleans, La.

Custodian, Magnus Pflaum, Pittsburgh, Pa.

Elective Members of the Executive Committee, Dr. W. W. Alleger, Washington, D. C., Dr. A. T. Kerr, Buffalo, N. Y., B. D. Myers, Ithaca, N. Y.

One of the most enjoyable features of the meeting was the informal reception tendered the society on Friday evening by Mr. J. F. Stone of Columbus. Mr. Stone showed a series of views of the Grand Canyon of the Colorado, made on his recent trip through the canyon in a boat. The views were magnificent, and were prefaced by a vivid account of the splendors of the scenery.

Several members of the American Microscopical Society waited for the meeting of the American Association for the Advancement of Science, and enrolled as members of the latter organization also.

Noticeable was the presence at Columbus of three original members of the organization, Dr. R. H. Ward of Troy, N. Y., its first president, Dr. G. W. Slocum of Marietta, O., and Dr. Edgar C. Taylor, familiarly known as "Gray-beard."

The society was indebted to Ohio State University for many courtesies extended to it, and to the local committee, under the chairmanship of Dr. A. M. Bleile, for the very satisfactory arrangements made to insure the success of the meeting. The trolley ride under the guidance of Mr. Feiel, another member of the local committee, was also thoroughly enjoyed. HENRY B. WARD.

University of Nebraska.

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METHODS OF STAINING.—The following methods are given by request :

*Stroebe's anilin blue stain for nerve fibers in hardened sections.*—Harden the tissue in Müller's fluid; stain one-half to one hour in a saturated aqueous solution of anilin blue; wash in water; transfer to a small dish of water containing twenty to thirty drops of a one per cent. alcoholic solution of caustic potash, (potassium 1; alcohol 100; let the mixture stand twenty-four hours and filter). In from one to several minutes the sections become bright brownish-red and transparent; place in distilled water five minutes; sections turn blue; stain in a half-saturated solution of safranin for a quarter to one-half hour; wash; dehydrate in absolute alcohol, and mount.

*Unna's polychrome methylen blue solution :*

Methylen blue, 1 part.  
Carbonate of potassium, 1 part.  
Distilled water, 100 parts.

This solution is oxidized for several months, when methyl violet and methyl red are formed. The ripened solution is sold by Grüber.

*Unna's modified orcein method :*

Grüber's orcein, 1 part.  
Hydrochloric acid, 1 part.  
Absolute alcohol, 100 parts.

The sections are put in a porcelain capsule with just enough of the stain to cover them, and warmed to about 30 degrees C. In ten or fifteen minutes the stain becomes thick, owing to the evaporation of the alcohol. The sections are then rinsed in alcohol, cleared and mounted. This method may be used for connective tissue. The elastin stains dark brown; the collagen, light brown.

*Phosphotungstic acid hæmatoxylin :*

Hæmatoxylin, 1 gr.

1 per cent. phosphotungstic acid (aqueous solution), 100 cc.

Heat the hæmatoxylin in a little water until it dissolves, and when cool add it to the dilute acid. The solution is greenish-brown at first, changing to pale brown. It may be used at once, keeps well, and requires no antiseptic. For elastic fibers, striated muscle fibers, fibrin, and cartilage, the tissue is hardened in alcohol, stained in the solution for twenty-four hours, washed in water, then dehydrated and mounted in the usual way. Nuclei and elastic fibers are stained blue; connective tissue, pink.

There are two neuroglia stains by Mallory—one with anilin-gentian violet, and the other, which is here given, with the phosphotungstic hæmatoxylin solution.

a. Fix in 4 per cent. aqueous solution of formaldehyde for four days.



*b.* Place in a saturated aqueous solution of picric acid for four days.

*c.* Leave in a 5 per cent. aqueous solution of bichromate of ammonium for from four to six days in an incubator, or from three to four weeks at room temperature. Dehydrate and imbed in celloidin. Stain in phosphotungstic acid hæmatoxylin twenty-four hours. Wash in water, dehydrate, clear in oleum origani cretici, and mount in xylol-balsam.

*Picric acid and acid fuchsin* (Van Gieson's stain).

For connective tissue :

1 per cent. aqueous solution acid fuchsin, 5 cc.

Saturated aqueous solution picric acid, 100 cc.

The stain is best for the coarse fibrillæ. It is sometimes better to increase the proportion of acid fuchsin. Fix the tissue in chrome salts or in corrosive sublimate; stain deeply in alum hæmatoxylin; wash in water; stain from three to five minutes in the picric acid fuchsin solution; dehydrate in 95 per cent. alcohol; clear in oleum origani cretici; mount.

For the nervous system :

1 per cent. aqueous solution acid fuchsin, 15 cc.

Saturated solution picric acid, . . . 50 cc.

Distilled water, . . . . . 50 cc.

Stain as for connective tissue. Nuclei are blueish red; ganglion cells and processes, red; axis-cylinders, brownish red; myelin sheaths, yellow; neuroglia fibers, red.

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Professor George W. Card, Geological Survey, New South Wales, writes from Sidney, as follows :

"It may interest you to know that additional evidence of the existence of elevated foraminiferal oözes in the South Pacific has come to hand from New Caledonia. Specimens of a limestone from Magenta, near Noumea, collected by F. D. Power, F. G. S., are of this nature. One specimen consists largely of a diversity of calcareous organisms, among which Nummuloid, Foraminifera, and Globigerina were conspicuous. In addition to numerous rounded fragments of other rocks, angular grains of quartz are present. Another specimen was an indurated semi-crystallized cream-colored limestone, breaking with an even fracture. Under the microscope this is seen to be a genuine Globigerina oöze consisting entirely of the remains of Globigerina and allied forms."

Professor Card enclosed an abstract of the meeting of the Linnean Society of New South Wales, held at Linnean Hall, Sidney, New South Wales, at which the following papers were read :

Contribution to Australian Ichthyology, by J. Douglass Ogilby. Notes from the Botanic Gardens, Sidney, by J. H. Maiden and D. Betcher. Description of a new Ophuiran, by H. Farquar. Dr. F. Tidswell gave a summary of what is known as "tick fever" in cattle, and in illustration exhibited a comprehensive series of preserved specimens, micrographs, and microscopical preparations of ticks, and of the tick fever, hæmatozoon *Pyrosoma bigeminum*.

# Journal of Applied Microscopy.

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## On Studying Slime Moulds.

### FIRST PAPER.

For readers of the JOURNAL, a discussion of this theme would seem less necessary after the very suggestive papers offered by Prof. Ayers last year. What I may say will, therefore, be considered simply as supplementary to the papers referred to, and will relate, in part at least, to phases of the subject less fully presented.

In the first place, then, I would emphasize the idea that the proper place to begin the study of these curious organisms, as indeed of living things generally, is *in the field*. He was a smart boy who, when asked where fossils were found, replied, "In the museum." His answer may not have been deficient in accuracy, but it certainly revealed a bad system of pedagogics. If we are to study nature to any purpose, we must go where nature is. True, we need laboratories and all appliances, but these come later; we should begin out of doors.

For Myxomycetes, then, I should first escort my pupil to the nearest bit of woodland, undisturbed. Not that woodland is indispensable; it is not. These things flourish too on the prairies, the plains, away out on the desert; but the woods are preëminently their home, and there the various phases of their history can be followed to highest advantage. Our undisturbed bits of primeval forest are fine, but the groves and thickets of the city park are sometimes equally productive. The lamented Dr. Rex, the foremost student of this group in the United States, if not in the world, found Fairmount Park, Philadelphia, an ample field for the labor of many years. Even the planted groves of our prairie farms, if not disturbed by cattle or other animals, afford habitat to some ubiquitous species, and will doubtless be richer and offer a more varied flora as the years go by.

In the woodlands, then, the Myxomycetes begin their activity about the first of June, in the latitude of our Northern states, and continue active, save as hindered by occasional drought, far into the autumn, sometimes in Iowa up to November 15. During all this time, especially in warm, wet weather, our student may find plasmodia,—free, raw protoplasm—streaming about to his heart's content. The amount discoverable in one place seems conditioned by the amount of nutrition available, and by that alone. An undisturbed old log, or a wide mat of decaying leaves, will often show plasmodia of surprising extent.

*Fuligo* is among the earliest, and its creamy, unctuous plasmodium may be found seated on stumps, or ascending to the height of several feet, the stems of trees, in quantity to be scooped up by the hand. On an old pine log in the Black Hills in South Dakota, I once came across the plasmodium of *Lindbladia effusa* (Ehr.) Rost, more than two feet in length and some six inches in width. Plasmodia of *Mucilago spongiosa* (Leyss.) Morg. are sometimes found in quantity to be lifted into a cup! For a student to come across such a mass of living matter, protoplasm if you please, almost undifferentiated, is interesting enough, and an experience never to be forgotten. The smaller venulose plasmodia which are during the season everywhere discoverable in their favorite habitats, are not less interesting; their peculiar appearance, form, color, are fascinating once the significance of it all is recognized. Very often the transition may be observed *in situ* from streaming plasmodium to perfected fruit. It is in many species effected in a few hours. At any rate the plasmodium may be properly marked and watched from day to day until life's round is finished. So much can be done in the field, and, as it happens, these are observations that just now are in most request. Of a comparatively small number of species is the plasmodium known. Such information must be gained, if gained at all, by the careful exactness of a multitude of observing students. Where continuous observation in the field is for any reason impracticable, a part of a plasmodium may be brought to the laboratory, where, in some cases, further steps in development may be watched. That fructification does not in such cases uniformly ensue, is perhaps due to the fact that the plasmodium, before fruiting, requires a certain maturity; where this is lacking the plasmodium in its changed conditions generally perishes. Plasmodia brought indoors should be kept in the dark and kept moist, though not too wet. As soon as signs of fruiting appear, the bell jar or other covering should be removed and the substratum may be allowed to dry.

Plasmodia in the field demonstrate their power of locomotion by the change in form and position which may be noted from time to time. Such plasmodia brought to the laboratory and placed on a moist glass in a moist chamber, as under a bell jar, will often leave the original substratum and be found on the glass. The latter may then be treated as a slide, placed on the stage of a microscope where the streaming of the protoplasm may be observed surging to and fro as long as proper conditions can be maintained. In this way we may discover that the color is due to the granular or floating contents of the protoplasmic stream, perhaps beneath our glasses disappears entirely. We may easily distinguish endosarc and ectosarc, and see that the translative movement is always initiated by the latter. Parts of the plasmodia may be induced to spread upon cover-glasses, to be subsequently fixed and stained. Alcohol as a fixing medium, followed by safranin or other anilin, will suffice to bring out the nuclei, and thus demonstrate the multicellular character of the one great amœba.

Another thing to be early noticed in the field is the effect of position and environment generally, upon the form and character which the fruit ultimately attains. For instance, the length or even presence of a stipe is sometimes due largely to the fact that the sporangia are formed on the lower and not on the upper side of the substratum, where gravitation contributes to lengthen out the

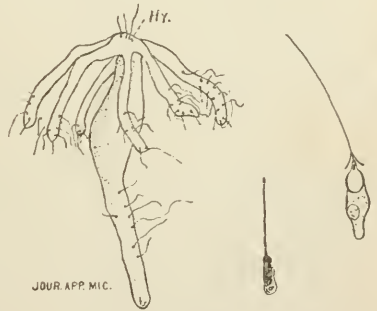
plastic stem. Of the general effect of varying atmospheric conditions also, the student will gain information in the field, and nowhere else. The marvelous work done by Fries in this group and among the fungi in general, was due to the circumstance that he studied these things in their haunts, and so, even without much aid from the microscope, as we count microscopes now, effected in classification much that will abide forever. On the further study of slime moulds in fruit we may speak later.

THOMAS H. MACBRIDE.

University of Iowa.

## On the Action of Methylen Blue on the Thread-Cells of Living Specimens of *Hydra Fusca*.

A few days since I felt desirous of testing the influence of methylen blue on living *Hydra*, and having a good supply of healthy specimens, made the experiment. I first made a weak solution of the stain by taking a small amount of the Bx stain of Gruebler and mixing it in hydrant water till the latter, passing through a faint blue color, reached a dark but still transparent tinge. I then dropped my *Hydra* in the solution and after a moment examined it with a low power. I was very much surprised to find that the tips of the tentacles were entangled with what seemed to be threads of wool. I could not see where the wool had come from, and I soon saw that it was not wool at all, but that at the base of each thread was a swelling imbedded in the ectoderm, and that the animal, perhaps from the irritation of the chemical, had discharged multitudes of the nematocysts, whose threads had at once become deeply colored. Soon this condition, which at first affected the tips of the tentacles, spread to their proximal ends, and affected also the body, threads being visible sticking out from the hypostome (Hy. in fig.) and also from the side walls.



Many of the threads were wholly separated from their insertion in the animal, and could be seen floating isolated in the water. Two kinds are clearly demonstrated by the method; viz., the larger barbed cells, and a second much smaller barbless kind. The two are also clearly visible *in situ* in face views of the tentacle, under the high power after staining. In these two types, I noticed that the thread is much thicker and shorter in the smaller type, and more delicate in the larger barbed kind. At the inner ends of both kinds of isolated nematocysts the remains of the cell are visible in many cases, and in this can be seen a well-stained nucleus.

H. L. OSBORN.

Hamline University, St. Paul, Minn.



## The Paraffin Method in Hot Weather.

It is frequently desirable, or even necessary, to prepare sections for examination during the heat of the summer months. Those who have tried to handle thin paraffin ribbons under such conditions of weather will appreciate the difficulties of the process. During such heated periods, I have been obliged at times to prepare slides, and driven to desperation by the unmanageableness of the thin paraffin ribbons, I have tried various plans to obviate the difficulties seemingly inherent in the methods. I was finally successful enough to obtain good results, and, in the hope of helping some one else, I will here give the simple means by which I operated. Most probably what I have to say will be anything but new to many, but possibly the methods may come as suggestions of value to some, and with that hope I will record them.

The great difficulty, of course, arises from the approach of the room temperature to that of the melting point of the paraffin, whereby the imbedding medium is rendered so soft as to be unable to withstand the impact against the knife, or to permit the handling of the ribbons without adhering to the instruments. Obviously, the only way to succeed in the treatment of such a substance is to render it cooler than the room; for it is not ordinarily convenient to lower the temperature of a room sufficiently, although this would be the more effective escape from the difficulties.

The means that render it possible to obtain good sections, even in the torrid heat of summer, are haste and ice judiciously administered. First I place the trimmed blocks of paraffin and their holders in a vessel containing cracked ice, and the knife upon a cake of ice, until all become thoroughly cooled. I then take a good sized crystallizing dish and fill it with small pieces of ice and place over it a pane of glass. With everything thus in readiness, I place the holders in the microtome, adjust the knife, and, as quickly as possible, cut the ribbons. These, as they come from the block, are laid along the knife until of sufficient length, when they are removed and spread upon the pane of glass over the ice.

No difficulty will be found in keeping the block and the knife cool in the more recent form of the Minot microtome, made in this country, where the knife rests in a horizontal position, for it is easy to run the block up to the knife and to lay a piece of ice upon the two. But in the older Minot with the knife set vertically, it is more difficult to keep the cutting instrument and the object to be cut in the proper condition of temperature. This, because the preliminary cooling will not be found sufficient in cases where the block is of some size, and several subsequent applications of the refrigerating agent therefore become necessary. However, by exercising a little patience, I have succeeded with both machines, and I am sure any one else can.

With the ribbons cut and spread on the cool glass, it will not be found difficult to divide them into appropriate lengths for mounting, whereas, if they were lying upon some substance at the temperature of the room, they would be found to stick in a most uncomfortable manner, not only to the support but to the cutting instruments. I found it most convenient to have the knife with which

the ribbons were cut into segments and the needles with which they were handled, as cool as possible, and for this purpose I kept them, when not in use, thrust down past the edge of the glass into the ice below.

If all the conditions are favorable, the sections should come from the knife with but few wrinkles, but in order to secure them absolutely free from any folds, I am accustomed to spread them by heat. To do this, I place the segments of ribbon upon a perfectly clean slip covered with a thin solution of albumen (three drops of Mayer's albumen to an ounce of distilled water). The whole is then placed upon the top of the paraffin oven where the temperature is below the melting point of the paraffin. Here, in a very short time, they extend their full length quite free from folds or wrinkles.

While thus warm, it would be quite impossible to arrange them on the slip, so the mount is again transferred to the cool pane of glass, and in this position, by the aid of some slips of absorbent paper, the sections of ribbon are established in position and the excess of fluid removed. Sometimes it is more convenient to manipulate the slides upon a Syracuse watch-glass, in the cavity of which are contained fragments of the ice. Should the little ribbons manifest a stubborn tendency to assume the form of circles or segments of the same, a few cuts on the concave side, extending not quite across, will render it possible to straighten them out satisfactorily.

Nothing now remains but to place the preparations upon the top of the paraffin oven and leave them for a couple of hours in order to secure the adherence of the sections to the slip, when they are ready for the further processes of staining and mounting in the usual manner.

University of Kansas, Lawrence.

C. E. McCLUNG.

## The Concilium Bibliographicum of Zurich.

The Third International Congress of Zoölogy, meeting at Leyden, voted unanimously to found in Zürich, Switzerland, a central bibliographical bureau, to be intrusted with the preparation of an analytical card catalogue of the current publications in zoölogy, taken in the widest sense. In November, 1895, an office was opened in Zürich under the name Concilium Bibliographicum, and the practical work was begun. The initial expenses were borne by the director of the institute, but for the current expenses dependance had to be placed upon grants that were made by the Swiss authorities, the American Association for the Advancement of Science, the Elizabeth Thompson fund, the American Microscopical Society, the Naples Zoölogical Station, the Société zoölogique de France, and by numerous generous donors in France, England, and elsewhere.

For several years past, the project has been actively discussed in the scientific press, and it was hoped that the Concilium would serve as a scientific agency similar to a mercantile bureau of information, so that a person desiring information in regard to some detailed question might obtain promptly full information in regard to the bibliography of that question. As will be seen in the sequel, the Concilium has remained faithful to this programme and indeed has already for the greater part accomplished this task.

For the first year, however, such action would have been impossible even under the most favorable circumstances, for the first requisite was to gather the information. Therefore, at the start, certain general groups were established for subscription, but it can hardly be said that individual questions were offered.

This first year proved, in reality, however, quite disastrous. The director of the Concilium was taken seriously ill at the beginning, and was for a long time unable to take any active part in the work. Furthermore, grave technical difficulties had arisen, which made it necessary to suspend publication and modify the plans in accordance with the experience that had been acquired. These difficulties were inherent in the card system, which had never before been put into use upon such a scale. Not being able to expect of our subscribers that such an enormous bulk of cards would be kept in order by a trained scientist, it was necessary to print symbols on the cards showing the order in which they were to be placed in the catalogue. For this purpose the Dewey decimal system seemed to us, on the whole, the simplest and most efficacious means; we have never regretted our decision in this regard. We found, however, that the application of the system to cards involved special difficulties which we had not foreseen. For an ordinary bibliography in pamphlet form, appearing at frequent intervals, a simple division into general chapters is quite sufficient. Not so with the cards; a division which in an ordinary monthly issue would only contain ten titles, would yield 120 cards by the end of the year, and in a few years would be quite unwieldy. In view of this cumulative character of the cards, the system must be very detailed. Moreover, while a book-bibliography publishes the titles in a given order adapted to the most general needs, the card system should strive to meet varied individual wants.

In no group of sciences are these individual needs so diversified as in Biology. One investigator wishes to know what has been published in regard to a given species, another wishes to follow a certain phenomenon through the entire animal kingdom and has little concern about the particular species that has served as material for study, yet another wishes to work up the fauna of a given district and requires a geographical arrangement, another cares less about the results than about the technical methods that have been used in the investigation. Hitherto, the zoological bibliographies have chosen one of these aspects alone; this is also the course that the Concilium had thus far followed. It was evident that if some means could be devised for permitting great freedom of arrangement without sacrificing the principle that the arrangement of the cards was to be purely mechanical, the new publication would take at once the highest rank. The difficulties, however, long seemed almost insurmountable.

In the spring of 1897, the problem had been practically solved, and publication was resumed. The results were not, however, fully satisfactory. The great fault lay in the fact that the same cards were to be used by subscribers in very different ways according as they wished to emphasize the systematic, the topical, or the faunistic aspect. This difficulty was very fundamental, since no printing office, save one especially equipped for the work, could well undertake the complicated operation of changing the symbols during the printing, so as to

supply a few cards for the one need, a few different ones for another special requirement.

This then led to the final step in the reorganization of the work. In the spring of 1898 a complete printing office was established in a house close to the offices\* and special equipment was secured so as to permit the printers to interchange certain symbols without removing the frame from the press. Thirty-five cards are printed at a time on large sheets, which are afterwards cut up and passed through the punching machine. They then come into the store-room and when a sufficient number have accumulated, pass on to the distributing room. Here the sorting is done by young girls by means of a multiple check system, which removes all chance of error.

Now that the system is in perfect working order, it is interesting to note how the single intellectual operation of assigning a given number to an article in a scientific journal causes the card representing that article to fall into the same identical place in card catalogues scattered all over the civilized world, from Christiania to Cape Town, and from Honolulu to Vienna. Indeed, no manuscript is prepared for the type-setters. Each paper is studied carefully, many are read entirely, and a simple note is slipped into the book indicating the contents, the new species, etc., and showing by simple signs the various uses that are to be made of a given title. The type-setters are chosen from different nationalities, so that all the modern languages can be dealt with. According to linguistic aptitude, the type-setters are given the publications themselves and compose the references according to certain definite rules of citation. In certain simple cases the specialist charged with the indexing would write merely a number on the cover of the pamphlet, 57.99 (74.7) for instance, and as a result, the announcement would be sent out that a new genus of bees had been described from New York State. This information would be given on a card satisfying all the rules of citation; it would also appear in its proper place among the Apidæ, and yet all the subsequent operations would have been carried out by persons entirely ignorant of the very existence of the group Apidæ.

The principle of classification is that in every case two aspects come into play and are given a varying value in the classification, according to the use to which a given card is to be put. Thus, in the case given above, the card would be printed for one subscriber in the order Apidæ—New York, for another in the order New York—Apidæ. This interplay of two or more aspects gives the whole system an adaptability which had long been sought but never before attained. At present there are 170,000 such groups in the scheme of classification, not to mention the use that is made of the alphabetical arrangement of genera within a given family. At first sight this statement would seem to forebode a terrible complication in the symbols, but this is far from being the case. Indeed, the users of the bibliography would be themselves the first to be astounded by this bit of statistics. Properly speaking, the symbols play the same part that do the page numbers of a book, the alphabetical key representing the index, and the methodical key the table of contents.

\*They have since been brought under one roof in the new and more commodious quarters of the Concilium.



At the close of the second year, the Concilium had published almost no anatomical and physiological titles, and the zoölogical references were sadly incomplete. Indeed, for the two years, not half of the requisite number of titles had been put through press. Since the establishment of the Concilium's printing office, this has been changed; in 1898, many of the back references were issued, and the present year will probably bring the work nearly up to date. The task of the year 1900 will then be to secure the greatest possible promptness.

The question now arises as to how far the Concilium can fulfill the task of affording investigators answers to definite bibliographical questions that they may wish to submit to it. This it is now certainly in a position to do. For the past two years it has received subscriptions to any division of its catalogue. A person subscribing for references to works on the fauna of the District of Columbia would evidently not receive many cards in a year, but the subscription would be opened whenever desired and such cards as might appear would be sent to him precisely as if he had taken the complete set of cards.\* From a financial standpoint this may be unwise, but it was felt that the disinterested character of the work demanded the sacrifice.

In closing, a word may be said in regard to the support that zoölogists and microscopists can give us. It is to be remembered that the work is not a commercial enterprise, but one of self-sacrifice of the severest kind. Thus far, there has always been a deficit beyond that covered by the subsidies received. According to the regulations, no profit will be allowed in the future. Should such a change in the finances come, the price will be reduced or the reserve used for improving the service. The enterprise certainly needs more subscribers, but above all, it wishes to maintain coöperative relations with the scientific world, so that it shall be the constant preoccupation of all those publishing zoölogical contributions to ensure their reaching the central recording agency. Were this custom to become general, it might become the natural corollary of publication, so that no one would think of describing a new species without recording the description in the Concilium. When that should have arrived, the priority difficulties would all be cleared away. It is unfortunately true that the publications of numerous American societies do not reach Zürich. We wish we could induce all to feel that the Concilium Bibliographicum is *their work*, to which they owe allegiance. The trifling sacrifice to each society would be indeed small compared with what others have done and are doing for the work.

Zürich.

HERBERT HAVILAND FIELD.

At a conference of delegates from American and European scientific societies, held at Wiesbaden October 10th and 11th, plans were made to found an international union of the principal scientific and literary bodies of the world. The object of the new society will be to initiate and promote scientific enterprises of general interest, and to facilitate scientific intercourse between different countries. It is to be known as the International Association of Academies. The first general meeting will be held at Paris next year.

\*The prices are reckoned by the number of cards sent, and vary according to the size of the order, from one-fifth of a cent to one cent a card.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

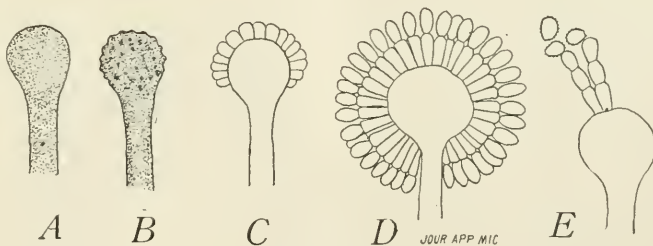
## IX.

## ASCOMYCETES.

This group, popularly known as the "sac fungi," contains an immense number of saprophytic and parasitic forms. Yeast, green mould on cheese and leather, leaf curl of peach, black knot of cherry and plum, and the powdery mildews are familiar to everyone. The few objects selected will enable the student to experiment, but he must not be discouraged if success does not crown the first attempt, for the group presents many difficulties.

*Saccharomyces*.—Until somewhat recently it was considered rather difficult to demonstrate the nucleus of the yeast cell. With fresh growing yeast, the following method by Wager should be successful. (See also the January, 1899, JOURNAL.) Fix in a saturated aqueous solution of corrosive sublimate for at least twelve hours. Wash successively in water, thirty per cent. alcohol, seventy per cent. alcohol, and methyl alcohol. Place a few drops of alcohol containing the cells on a cover, and when nearly dry add a drop of water. After the yeast cells settle, drain off the water and allow the cells to dry up completely. Place the cover, or slide, with its layer of cells in water for a few seconds, and then stain with a mixture of fuchsin and methyl-green, or fuchsin and methylen blue. Mount in glycerine or in balsam.

*Eurotium*.—For class use or for permanent preparations it is best to select

Fig. 28. *Eurotium*.

From material growing on a hectograph pad. Fixed in chromo-acetic acid, stained in eosin, and mounted in glycerine. A-E successive stages in development. > 375.

rather young material which shows various stages in development, from the swollen end of the hypha to the ripe spore. The nuclei are exceedingly small, and can hardly be demonstrated with eosin. Iron alum hæmatoxylin would be better. A saturated solution of corrosive sublimate in fifty per cent. alcohol, used hot, can also be recommended for fixing. Mount in glycerine. For paraffin sections use the safranin-gentian violet-orange combination, and mount in balsam.

Other filamentous forms, like *Penicillium*, may be treated in the same way.

*Uncinula necator*.—The mildews are found throughout the summer and autumn on the leaves of various plants. The lilac mildew (*Microsphaera alni*) and the mildew on the Virginia creeper (*Uncinula necator*) are particularly abundant. For herbarium purposes they may be preserved by simply drying the leaves under light pressure. When mounted for examination, the leaf should be soaked in water for a few minutes, after which the perithecia may be scraped off and mounted in [water. The asci may then be forced out by pressing smartly on the cover.

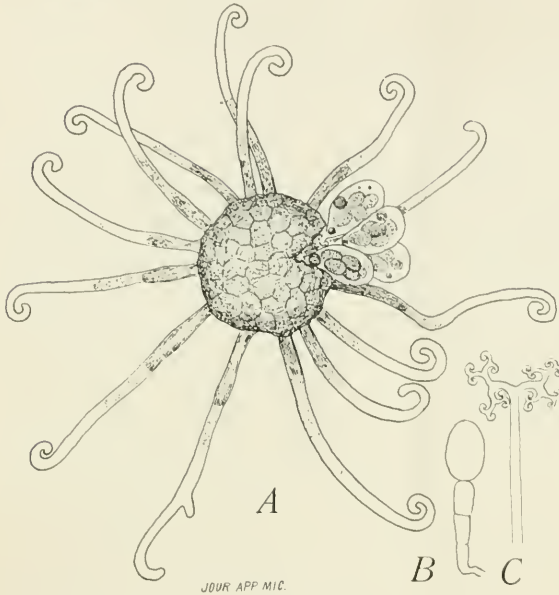
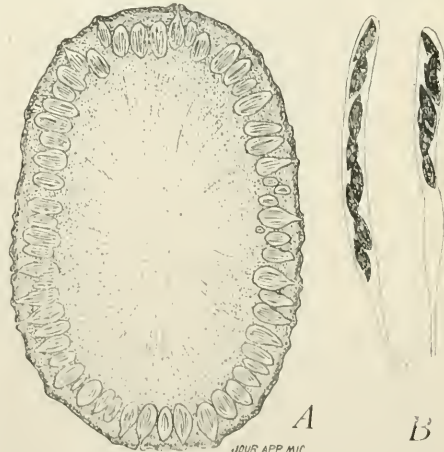


Fig. 29.

A. *Uncinula necator* on *Ampelopsis quinquefolia*. 192. Four asci containing ascospores have been forced out by pressing on the cover. Fixed in hot corrosive sublimate, stained in fuchsin and mounted in balsam. B, a conidiospore; and C, an appendage of *Microsphaera alni*, drawn from living material. 192.

per cent. formalin twenty-four hours, wash in water one hour, stain in aqueous eosin twenty-four hours, treat with one per cent. acetic acid one minute, wash thoroughly in water, and then transfer to ten per cent. glycerine, which should be allowed to concentrate as usual. If chromic acid or corrosive sublimate be used for fixing, the appendages become brittle, and very easily break off. However, the chromo-acetic mixtures are better if it is desired to make paraffin sections showing the development of the perithecium with its asci and spores. For this purpose the omnipresent *Erysiphe commune* on *Polygonum aviculare* is exceptionally favorable because, after the material is fixed and in alcohol, the whole mycelium, with the developing perithecia, may be stripped from the leaf without the slightest difficulty, thus avoiding the necessity of cutting the leaf in order to get the fungus. Delafield's hæmatoxylin gives good results. A

Fig. 30. *Nylaria*.

A. Transverse section of a young stroma showing perithecia. 8. Fixed in chromo-acetic acid, stained in bulk in alum carmine, imbedded in celloidin, and mounted in balsam. B. Two asci with spores. 245. The mature stroma was soaked for several days in equal parts of 95 per cent. alcohol and glycerine, and then imbedded in celloidin. Not stained.

mixture of acid fuchsin and methyl green or the safranin-gentian violet-orange may be tried for nuclei.

*Xylaria*.—Many forms, like *Xylaria*, *Ustilina*, *Hypoxylon*, and *Nummularia*, in their mature condition, are woody and so extremely brittle that it is almost impossible to cut them. As good a plan as any seems to be to cut sections of the stroma about one-eighth of an inch thick, soak them in equal parts of glycerine and ninety-five per cent. alcohol, and then imbed them in celloidin in the usual way. They might be cut without imbedding, but most of the asci and spores would then be lost.

The younger stages, showing the development of perithecia and asci, are more interesting, and can be cut in paraffin and stained with ease.

*Peziza odorata*.—The *Pezizas* and related forms are fleshy and present but little difficulty in fixing, cutting, or staining. They are abundant in moist places on decaying wood or on the ground. The apothecia have the form of little cups, which are sometimes black and sometimes flesh colored, but often orange, red, or green. For the development of ascospores in the ascus Flemming's fluid (weaker solution), followed by safranin-gentian violet-orange, has given the best results with thin sections where the mitotic figures are to be studied. Cyanin and erythrosin is also to be recommended. For a general morphological preparation, such as is shown in the figure, it is better to stain in bulk in alum carmine or in Delafield's hæmatoxylin, and then tease out the asci in glycerine or balsam. Sections thick enough to show the entire ascus are not usually as satisfactory as such teased preparations.

#### AECIDIOMYCETES.

The Aecidiomycetes comprise the rusts (*Uredineæ*) and the smuts (*Ustilagineæ*).

*Puccinia graminis*.—The common rusts of wheat and oats are familiar to everyone. The uredospores or summer spores, known as the red rust, and the teleutospores (last spores) or winter spores, known as the black rust, are found in unfortunate abundance, but the æcidium stage on the barberry is not necessary for the vigorous development of rust in the United States, and is seldom found. Most teachers are obliged to depend upon botanical supply companies for this material. There are, however, various æcidia which are as good, or even better, for morphological study. The æcidia growing on *Euphorbia maculata* (spotted spurge), and on *Arisæma triphyllum* (Jack-in-the-pulpit) are much easier to cut and seem easier to stain. Delafield's hæmatoxylin is good for both æcidia and spermatogonia, especially after Flemming's fluid. It is rather difficult to get good sections of uredospores and teleutospores, because the leaves of

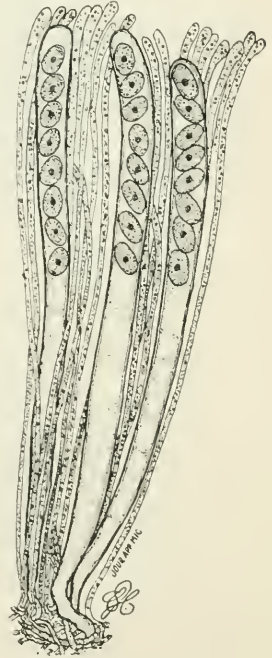


Fig. 31. *Peziza odorata*.  
Three asci and many paraphyses.  
245. Fixed in corrosive sublimate, stained in bulk in alum carmine. Teased out and mounted in balsam.



wheat and oats are refractory objects to cut. The cutting is easier after picro-acetic acid than after corrosive sublimate or the chromic acid series.

Every class which studies the rusts should attempt to germinate the uredospores and teleutospores. For this purpose the hanging drop culture may be

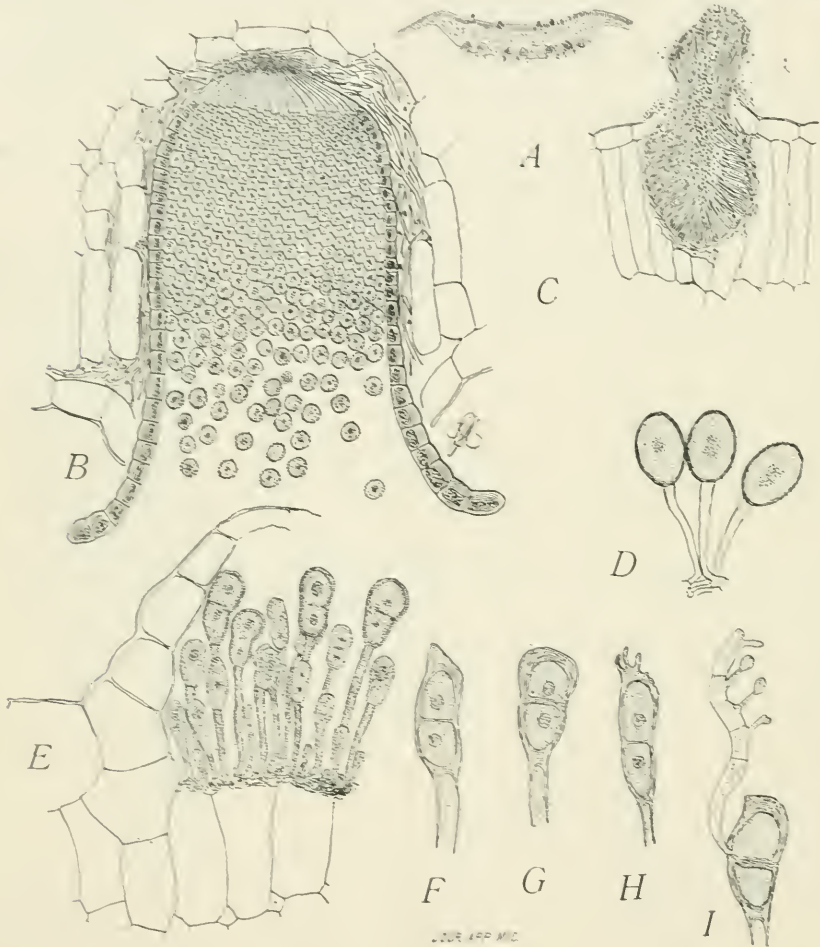


Fig. 32. *Puccinia graminis*.

- A. Transverse section of Barberry leaf showing acidia and spermatogonia.  $\times 7$ . B. Longitudinal section of a single acidia. 102. Fixed in Flemming's weaker solution and stained in Delafield's haematoxylin. C. A single spermatogonium. 102. Fixed and stained as in B. D. Three uredospores growing on oats. 375. Fixed in two per cent. formalin, stained in bulk in alum carmine, and teased out in glycerine. E. Section of young teleutospores on oats. 375. Fixed in picro-acetic acid and stained in cyanin and erythrosin. G, F, H. Three ripe teleutospores from a leaf of oats showing variation in form. 375. I. Germinating teleutospore. 375.

employed. Cement a rubber or zinc ring to the slide, or simply smear the lower surface of the ring with vaseline and press it tightly against the slide: smear the upper surface of the ring with vaseline, and over it invert the cover-glass with a shallow drop of water containing the spores. (Fig. 33.) The uredospores germinate readily all summer, but it is said that the teleutospores will germinate

only in the spring following their maturity. However, the teleutospores of many species, like *Puccinia xanthii* on *Xanthium canadense* (Cocklebur), will germinate as soon as they ripen and will serve equally well for study. If a particularly good specimen is secured, it may be preserved by the method previously described for desmids, except that in this case it might be worth while to attempt staining with Mayer's hæmalum, or with eosin.



Fig. 33.

The smuts may be studied in the living material. The method recently described by Ellis is worth remembering.\* A supply of smutted barley may be obtained by sowing soaked, skinned barley that has been plentifully covered by *Ustilago* spores. In such material it is easy to trace stages in the development of spores. Free-hand sections of ears about three-eighths of an inch long show the mycelium and spore clusters. If smutted ears be removed and kept floating on the water, the spores continue to develop and often germinate. For paraffin sections desirable stages should be fixed in Flemming's fluid or picro-acetic acid. Delafield's hæmatoxylin, followed by a very light touch of erythrosin or acid fuchsin, will give a good stain.

#### BASIDIOMYCETES.

This is an immense group of which the mushrooms, toadstools, puffballs, and bracket fungi are the most widely known representatives.

*Coprinus comatus*.—This is the common shaggy-mane mushroom. Cut from the cap pieces about one-fourth of an inch square, and fix in chromo-acetic acid or in Flemming's fluid. Portions in which the gills have just begun to turn brown will show the spores still attached to the sterigmata. If the gills have become dark brown or black, the spores will wash off before the sections can be mounted. Look in portions in which the gills are still white or only slightly changing color for the development of basidia and spores. The nuclei, although rather small, are brought out nicely by safranin-gentian violet-orange. The same procedure may be observed for other forms of similar consistency, like many members of the genera *Boletus*, *Hydnum*, *Polyporus*, *Lycoperdon*, etc. Leathery or woody forms like *Stereum* and many species of *Polyporus* had better be fixed in picro-acetic acid and imbedded in celloidin. Young stages of *Cyathus* (birds-nest fungus) cut easily in paraffin, but the older stages cut much better in celloidin. It is hard to get the very soft, watery forms like *Tremella* into paraffin without shrinking, but sections as thin as  $10\ \mu$  may be cut in celloidin. While this is too thick to give satisfactory views of such small nuclei, it brings out very clearly the general morphological structures.

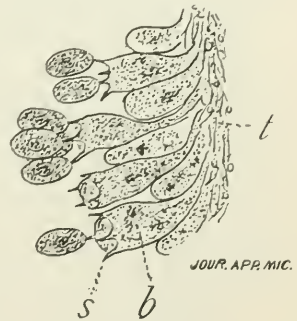


Fig. 34. *Coprinus comatus*.  
Transverse section of a portion of one of the gills showing a part of the trama, *t*, and several basidia, *b*, each with four sterigmata, *s*. Spores have fallen off from many of the sterigmata.  $\times 750$ .

\* Ann. Bot. 12: 566-567, 1899. Also Jour. App. Mic. 2: 254, 1899.

## THE LICHENS.

Some of these forms may be cut in paraffin, but most of them are so hard or leathery that better results may be secured with celloidin. All of the lichens should be fresh and moist when they are put into the fixing agent. Wherever difficulty is anticipated in the cutting, picro-acetic acid may be recommended for fixing and usually, celloidin for imbedding. The younger apothecia of forms like *Physcia*, *Usnea*, *Sticta*, and *Peltigera* cut well in paraffin, but results are uncertain with the older apothecia.

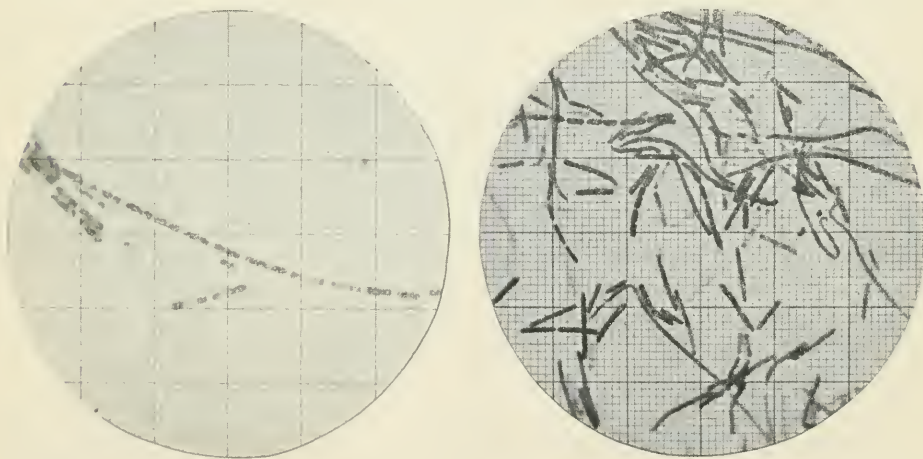
(To be continued.)

## Bacterial Measurements.

The method here described has been in use in this laboratory for the past two years, and has been found a simple, accurate, and satisfactory means of measuring bacteria and of recording those measurements.

The photographic apparatus is so adjusted that an amplification of one thousand diameters is secured. This is determined by measuring the magnified image of a stage micrometer on the ground glass screen.

A drawing is made of a convenient size, by ruling, with ink, two sets of



equidistant lines at right angles to each other, each tenth line being somewhat heavier than the others; this drawing should be at least four times the size of the negative to be prepared from it, in order to secure the requisite fineness of the lines. This drawing is then reduced by photography to such a size that the rulings are exactly one millimeter apart. The negative so obtained is the scale used.

The image of this scale is superimposed on the image of the photo-micrographic negative by a process of double printing, the photo-micrographic negative being printed first, and the scale afterwards on the same paper, or *vice versa*.

The amplification being one thousand diameters and the scale being in millimeters, the reading is directly in micro-millimeters.

The accompanying photo-micrographs will demonstrate better than any description, the value of the method.

E. H. WILSON, M. D., Director.

R. B. F. RANDOLPH, A. C., Associate Director.

Department of Bacteriology, Hoagland Laboratory, Brooklyn, N. Y.

### An Apartment Incubator for Student Use.\*

One of the difficulties in teaching bacteriology in the laboratory to a large class of students, is the procuring of sufficient incubator room. In our elementary course it has been found necessary for each student to have considerable incubator shelf space for his various cultures, including boxes for holding test-tube cultures, Petri dishes, and fermentation tubes. An area of about

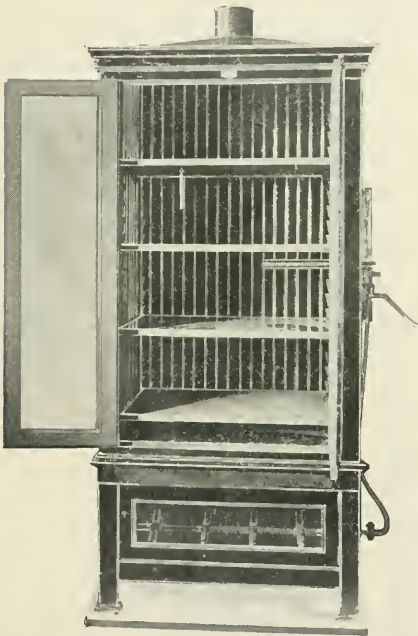


Fig. 1.

A photograph of incubator as originally built, Weisnegg pattern.

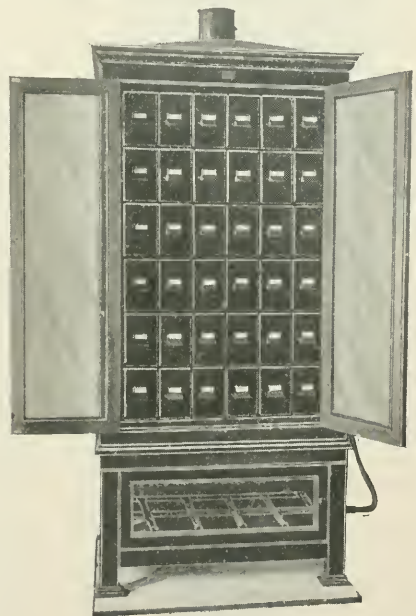


Fig. 2.

A photograph of incubator with apartment drawers.

eighty square inches has been found to be the minimum space which can be assigned to each. In fitting up our laboratory for students this difficulty was in a measure anticipated, and two large incubators built on the Weisnegg pattern were provided. These were found to afford ample shelf room for our present class, but the difficulty of utilizing the rear half of each shelf soon became

\*Presented at the meeting of the American Microscopical Society, Columbus, August, 1899.



apparent. The shelves were necessarily too close together to enable one to easily reach over the cultures standing in front, and the result was that, notwithstanding our cautioning and the intelligent care on the part of the student, it not uncommonly happened that cultures were misplaced, or, worse still, pushed from the shelves to the floor with the attending consequences.

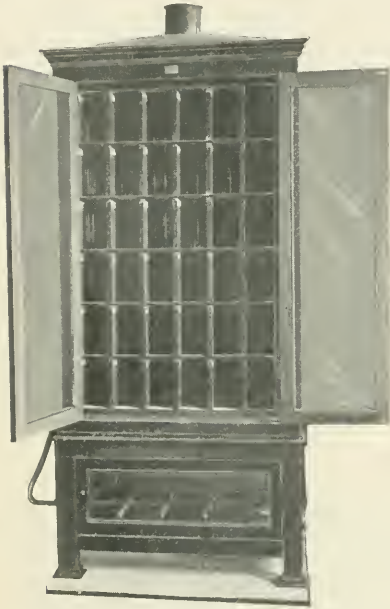


Fig. 3.

A photograph of incubator with framework for drawers.

In order to overcome the confusion, accidents, and annoyance to students where cultures were misplaced, or perhaps destroyed by others in removing those in the rear, the desirability of constructing individual apartments suggested itself. A number of devices were considered, but the one about to be described seemed, all things considered, to be the most practicable. It consists simply of a chest of drawers somewhat on the Lillie water-bath pattern, placed within the incubator, each drawer being of sufficient size to furnish storage for the working cultures of one student. This removes all possible excuse for meddling with the cultures of others, and the drawer also affords a convenient tray in which to transfer cultures from incubator to work table and *vice versa*.

As already stated, we had the large Weisnegg form of incubators. These are heated by the radiation from a metal plate at the top and one at the bottom,

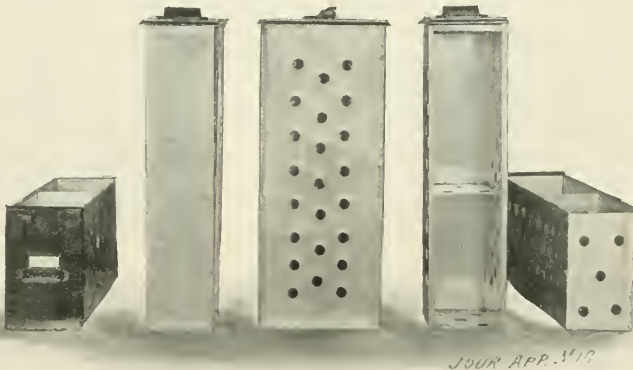


Fig. 4.

A photograph of incubator drawers showing different views.

and metal tubes connecting them. The tubes are arranged at the two sides and back and are placed close to each other (see Fig. 1). This arrangement gives

lateral heat quite as much as a water-jacketed incubator. The shelves with the standards supporting them were removed, and in their place the framework for the drawers was fastened (Fig. 3), leaving a narrow space on all sides.

The drawers are made from sheet zinc with a wooden front. Each drawer is 49 centimeters long, 10.5 centimeters wide, and 19 centimeters deep. The perforation at the sides and rear end allows quite free passage of air. The ends are soldered, and the perforations are sufficiently high from the bottom for the drawers to hold the cultures, if for any reason the tubes should break. The drawers can be easily sterilized.

The board front permits of the closing of the front of the incubator, so that the opening of the door affects the temperature but very little. The sides of the drawer are provided at the top with a narrow flange which runs in a metal groove and in which the drawer is supported. The grooved strip is imbedded in the framework. On the front end of the drawer is an inexpensive and convenient pull, which is also provided with a frame for a card on which to write the name of the student using the drawer.

In regulating these we use the Roux bimetallic regulator, which is inserted in the space at the back rather than at the side, as shown in Fig. 1. The temperature does not vary more than a degree in the different drawers. The size of the drawers would possibly be better if a trifle larger, but we were obliged to use the incubators already built, and also to provide for the maximum number of students, and consequently each drawer contains the minimum area. I am indebted to Mr. Henry Bool, who built the drawers, for his skill in minimizing the space occupied by the framework, and to Mr. Raymond C. Reed of this department for the photographs.

VERANUS A. MOORE.

Department of Comparative Pathology and Bacteriology.  
New York State Veterinary College, and Cornell Medical College, Ithaca, N. Y.

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The first report on the study of malaria, made by Dr. Koch, has been published in the *Deutsche Medizinische Wochenschrift*. The parasite of malaria was found in the human blood and in certain species of mosquitoes. Professor Koch identifies the æstivo-autumnal fevers with tropical malaria.

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English teachers in the vicinity of the Whitechapel Public Library and Museum use the natural history collections for illustrating object lessons. A list of the lessons as planned is sent to the curator of the museum, and the material required for the demonstration is placed on tables to which the students are allowed access. In this way it is possible for them to give the specimens close study in connection with their regular work. The suggestion might be of value to teachers and curators of museums in this country; collections that have been studied in this way have a much greater meaning for the student than those that may be observed only in glass cases, and without a special study of important features and relations of different forms.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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A CORRESPONDENT recently said: "Why not publish some elementary papers that the beginner can understand and which will not require a college laboratory equipment to follow out?"

Expressions similar to this have reached us at intervals during the year.

Careful investigation as to the causes which give rise to them has led to the following statement for the guidance of beginners in the use of the microscope, who look to the JOURNAL for assistance in their work.

It is our purpose to publish a certain amount of material for the beginner, and we have done so from the initial number. The series of papers by Dr. Huber, Dr. Novy, Professor Schaffner, Professor Chamberlain, and single papers by many others are of this character.

These articles differ from much that has heretofore been published, ostensibly for the beginner, in that they deal only with methods and apparatus; what to do, how to do it, and what to do it with, omitting entirely the sentimental or ideal side of the subject, which we believe has no place in a publication such as this.

Careful inquiry has elicited the fact that some have been deterred from even giving the papers referred to a careful reading because they are written by college men and deal with processes which have been developed in their laboratories.

While this is true, the ingenuity of the average man will enable him to duplicate with simple devices of his own manufacture all, or very nearly all, that is required to follow out the directions given, satisfactorily, and to prepare the reagents and material actually needed.

The beginner is often not satisfied to *begin far enough back*. It is manifestly useless to seek a method for demonstrating chromatophores unless one knows the meaning of chromatophore, and yet many beginners are wont to commence in the middle of the book, neglecting the preparatory chapters which would make the succeeding ones plain. The JOURNAL has elementary chapters in *methods*, but does not reach out into the rudiments of general biology.

Our idea of the right kind of a paper on methods for the beginner is one that begins at the beginning and explains each step in the order in which it would naturally occur in the course of work, leaving nothing ambiguous and presupposing no previous knowledge of the subject in question.

Papers redundant with generalities are easily prepared and of little value, although to the beginner often more interesting to read. Such papers have their function, but are not reliable guides for work.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

Davis, B. M. The Spore-Mother-Cell of Anthoceros. Bot. Gaz. 28: 89-109, pl. 9-10, 1899.

Anthoceros is a remarkably favorable form for studying a close series of stages in the development of spores from em-

bryonic tissue, since in a single longitudinal section of a sporogonium one finds the archesporium passing by insensible gradations into the mother-cell which furnishes an equally close series leading up to the mature spore.

The chloroplasts were found in the archesporium, these structures not being differentiated until the mother-cell stage was reached. The chloroplast first appears as a slightly denser portion of the protoplasm, but the first clear proof of its presence is the sharp staining of starch grains in its interior. As the chloroplast develops, its outline becomes distinct, but no surrounding membrane can be distinguished. The division is by simple constriction, the two new chloroplasts passing to opposite poles of the elongated spore-mother-cell, where they again divide. After its first appearance, the quantity of starch steadily increases until in the oldest spore-mother-cells the chloroplast seems little more than a storage vesicle of starch.

The nucleus of the spore-mother-cell passes into synapsis soon after the first division of the chloroplast. This phenomenon is not regarded as an artifact. The spindle of the first division seems to be formed about as described by Mottier (pollen-mother-cells of *Lilium*) and Osterhout (spore-mother-cells of *Equisetum*). After the first division the nucleus passes into a resting stage. The structural features of the second division are identical with those of the first. The number of chromosomes in the antheridium and in the spore-mother-cells is four, and in vegetative cells, eight. The splitting of the chromosomes seems to be longitudinal.

The mature mother-cell contains four chloroplasts, each with a single nucleus on its anterior side. The walls separating the mother-cell into four spores are derived from films of protoplasm which appear between the chloroplasts. These films are formed by the coalescence of strands of protoplasm which cross the space between the four regions of cell contents. No connection was established between these strands and the spindle fibers. These results do not agree with Farmer's accounts of nuclear division in *Pallavicinia*, *Aneura*, *Fossombronia*, and *Pellia*, but *Anthoceros* differs so widely from members of the Jungermanniaceæ that the differences need not occasion surprise.

It would seem to the reviewer that this work, supported by such critical preparations, would have justified Dr. Davis in attacking the permanent organ theory of the chloroplast. However, he promises to make a further examination of this subject.

The paper is accompanied by two excellent plates.

C. J. C.



**Belajeff, W.** Ueber die Centrosome in den spermatogenen Zellen. Ber. d. deutsch. bot. Gesell, 17: 199-205, pl. 15, 1899.

Several months ago Belajeff expressed the belief that the deeply staining bodies described by himself in the spermatogenous cells of *chara* (1892) and in the Filicineæ and Equisetaceæ (1895) represented the centrosome, and he claimed the same homology for the conspicuous bodies in the spermatogenous cells of *Cycas*, *Gingko*, and *Zamia* as described by Hirase, Ikeno, and Webber. He also believes that Shaw is incorrect in not regarding the blepharoplasts of *Onoclea* and *Marsilea* as centrosomes. The present paper is accompanied by a plate showing centrosomes in the mother-cell and grandmother-cell of the spermatozoids of *Gymnogramme sulphurea*, *Marsilea macra*, and *M. Vestita*. These centrosomes would, of course, be called blepharoplasts by Webber and Shaw.

In conclusion, the question is asked why it is that we do not find such structures in vegetative cells of vascular Cryptogams and Phanerogams. The answer suggested is that the dynamic center, which we call a centrosome, may be present in all cells, but may not always contain a substance sensitive to stains which would make it visible in preparations.

C. J. C.

**Němec, B.** Zur Physiologie der Kern- und Zelltheilung. Bot. Centralbl. 77: 241-251, 7 figs., 1899.

This paper contains a further exposition of the writer's views in regard to the differences between the mitoses of reproductive and vegetative cells. The general conclusion is that in relatively free-lying cells of the sporogenous tissue the achromatic figure is multipolar and often originates radially around the nucleus, while in cells of the vegetative tissue the achromatic figure is bipolar from the beginning. In vegetative nuclei the spindle makes its earliest appearance as a pair of hyaline caps at opposite poles. No centrosomes were found with the vegetative nuclei even in those plants like *Equisetum*, *Cycas*, *Zamia*, and *Gingko*, which in certain reproductive cells have such conspicuous centrosomes. The list of plants examined includes forty-four species, fairly representing Pteridophytes, Gymnosperms, and Angiosperms.

C. J. C.

**Bruchmann, H.** Ueber die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien. Gotha, pp. 119, 7 pl., 1898.

This work supplies a long felt need, for few plant structures have been less accessible or less studied than the prothallia of *Lycopodium*. The prothallia of the European species are described in considerable detail, and they present such marked characteristics that the writer classifies them under five groups; viz., (1) Type of *L. clavatum* and *L. annotinum*; (2) Type of *L. complanatum*; (3) Type of *L. selago*; (4) Type of *L. phlegmaria*; (5) Type of *L. cernuum*, and *L. inundatum*.

In all the types the antheridia and the archegonia are borne upon the upper surface of colorless prothallia, which, in some cases, become green if exposed to the light. In *L. selago*, and *L. phlegmaria*, there are paraphyses among the antheridia and the archegonia. In *L. selago* the prothallium produces adventitious branches.

The development of the embryo of *L. clavatum* is described in great detail. After fertilization the oöspore becomes pear-shaped, and then divides into a

larger suspensor cell facing the neck of the archegonium, and a smaller embryonal cell which divides into eight cells, the lower four producing the foot, and the other four the shoot and first root. Lateral roots originate from the cortex and not from the pericambium, as stated by Van Tieghem.

In discussing relationships the writer comes to the conclusion that *Lycopodium* should not be placed near *Selaginella*. The gametophyte bears some resemblance to that of the mosses, but the sporophyte is nearer that of the higher plants. The suggestion that *Lycopodium* should be broken up into several genera will probably meet with considerable opposition.

C. J. C.

**Fullmer, E. L.** The Development of the Microsporangia and Microspores in *Hemerocallis fulva*. Bot. Gaz., 28: 81-88, pl. 7-8, 1899.

It has long been known that in *Hemerocallis fulva* the mother-cell often produces more than the usual four microspores. When first described

the origin of the extra nuclei was attributed to a mitosis of one or more of the nuclei of the tetrad. More recently their origin has been attributed by Juel (*Cytologische Studien*) to chromosomes, which in some way have become isolated during one of the divisions of the spore-mother-cell. The present writer returns to the earlier view.

The tube nucleus frequently divides by direct division, sometimes giving rise to six or eight nuclei. This is the fourth plant in which such a division has been described, the previous cases being *Lilium tigrinum*, *L. auratum*, and *Eichhornia crassipes*.

C. J. C.

**Van Tieghem, Ph.** Structure de quelques ovules et parti qu'on en peut tirer pour améliorer la classification. Jour. de Bot., 12: 197-220, 1898.

In introducing the subject the complaint is made that in systematic works ovules are superficially described and inaccurately figured. The results

recorded here are based upon an examination of the ovules of twenty-six families of the *Seminatæ*, one of the two groups into which he divides the *Dicotyls*. The *Seminatæ* he divides into two classes, the *Crassinucellatæ*, or those in which the nucellus is rather thick and retains its form until the time of fertilization, and the *Tenuinucellatæ*, or those in which the nucellus consists of a single layer of cells. Each of these classes is subdivided into two orders, the *Unitegminatæ* and the *Bitegminatæ*, according as they have one or two integuments.

The investigations have brought us a large amount of information in regard to the ovule, but it is doubtful whether a classification based so largely upon one character will meet with any general acceptance.

C. J. C.

**Guignard, L.** Les centres cinétiques chez les végétaux. Ann. des Sci. Nat. Bot., Ser. VIII, 5: 178-220, pl. 9-11, 1898.

Various fixing agents were employed, but the best results were obtained with Flemming's fluid in the following proportions:

water 100 cc., chromic acid one-half g., osmic acid one-half g., and acetic acid two cc. The most satisfactory staining was secured by using a mixture of methyl green, acid fuchsin, and orange G in aqueous solution. The proportions should be such that, after about twelve hours, the chromosomes of

dividing nuclei appear dark green, the spindle a dark red, and the corpuscles at the poles dark green or violet, sometimes surrounded by an orange rose tint. Upon adding acid alcohol (100 cc. alcohol and 1 cc. HCl.), the excess of methyl green is removed in a very few seconds, the spindle takes on a brighter, purer color, and the corpuscles at the poles become more distinct. This combination gave better results than the popular safranin-gentian violet-orange, or the Heidenhain's iron alum hæmatoxylin.

Observations were made upon the two nuclear divisions, by which the four pollen grains are formed from the pollen-mother-cell in *Nymphaea*, *Nuphar*, and *Limnodorum*. In *Nymphaea* multipolar spindles are quite frequent, but they are not always followed by the bipolar stage.

The illustrations, which seem rather small and indefinite for such a disputed subject, show numerous centrosomes, some with and some without radiations. The occurrence of multipolar spindles is not considered a sufficient argument against the presence of centrosomes. The writer concludes that there are in the higher plants, differentiated centers, which function like the centrosomes of lower plants and of animals.

C. J. C.

**Hartog, M.** Nuclear Reduction and the Function of Chromatin. *Nat. Sci.*, **13**: 115-120, 1898.

After referring to the three modes of chromatin reduction, the writer suggests that the linin is the important substance, the fair and equal division of which is the final cause of karyokinesis. The splitting of a viscid thread presents great mechanical difficulty. If we suppose a polarity in the chromatin granules, which, after splitting, tend to recede from each other, the thread would be split and distributed. If we regard the linin as the transmitter of hereditary qualities, and ascribe to the chromatin granules a mechanical function, we avoid the difficulty encountered in attributing hereditary qualities to the chromatin, which is so subject to periodic growth and atrophy.

C. J. C.

**A Guide to the Study of Lichens.** Albert Schneider, M. D., Phil. D., Professor of Botany, Materia Medica, and Pharmacognosy, Northwestern University School of Pharmacy. Pp. XII, 234, with 11 plates. Bradlee Whidden, Boston, 1898.

Now that this book has appeared, there are very few, if any, things left which the rambler is likely to meet to which he cannot find a "guide," and if there be any group of organisms visible to the naked eye to which it would appear almost impossible to prepare a guide, it seems as though the lichens must be that group. When one considers that their distinctive characters are principally microscopic, the algæ and the spores, the difficulty of preparing a satisfactory guide, with analytical keys, which can be used by amateurs, becomes apparent. Nevertheless the author has made it perfectly possible for these persons to become familiar with the more common genera of the group through a study of their macroscopic features alone. Although many species are described, the keys extend only to the genera. If the student have a compound microscope he can, of course, make his identifications with much greater certainty, but in this case he will probably prefer to use the author's "Text-book of Lichenology," of which this guide appears to be a simplified condensation. About fifteen pages are devoted to a discussion of the

the naked eye to which it would appear almost impossible to prepare a guide, it seems as though the lichens must be that group. When one considers that their distinctive characters are principally microscopic, the algæ and the spores, the difficulty of preparing a satisfactory guide, with analytical keys, which can be used by amateurs, becomes apparent. Nevertheless the author has made it perfectly possible for these persons to become familiar with the more common genera of the group through a study of their macroscopic features alone. Although many species are described, the keys extend only to the genera. If the student have a compound microscope he can, of course, make his identifications with much greater certainty, but in this case he will probably prefer to use the author's "Text-book of Lichenology," of which this guide appears to be a simplified condensation. About fifteen pages are devoted to a discussion of the

history of lichenology, ending with an account of the views of Schwendener and Reinke, the latter of whom has the author's support in believing that lichens are morphological units and not symbiotic formations. The remainder of the first part of the book contains sections on the uses of lichens, their supposed mode of evolution, morphology and physiology, occurrence and distribution, and directions for collecting, preserving, and studying them. The second part consists of the systematic description. Appended sections give the continental range of the more important lichen genera occurring in the United States, and a list of all the species known to occur in the same region. The plates illustrate algal and lichen types and generic spore types.

This guide will undoubtedly do much to stimulate interest in lichens among the rapidly increasing numbers of amateur naturalists, few of whom suspect the curious symbiotic and ecological relationships exhibited by these plants.

CHARLES WRIGHT DODGE.

#### RECENT LITERATURE.

**Barnes, C. R.** The Progress and Problems of Plant Physiology. *Science*, 10: 316-331, 1899.

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**Buscalioni, L.** Osservazioni e ricerche sulla cellula vegetale. *Annuario del R. Istituto botanico di Roma*. 7: 255-346, pl. 8, 1898.

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#### ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

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#### CURRENT LITERATURE.

**Mayer, Paul.** Ueber Hæmatoxylin, Carmin und verwandten Materien. *Zeit. für wiss. Mikr.* 16: 196-220, 1899.

Several years ago the author was asked to write an article on stains for a handbook to be used by botanists, zoölogists, and mineralogists. The work was never completed, but in this full and complete article the same ground is covered. Before starting the treatment of the subject proper, a short explanation is given of "stains" and "mordants" and the parts played by each in finally staining tissues. They do not, he says, stand in sharp contrast with each other, but conditions will make the one take the place of the other. Plant or animal tissues can be saturated with an alum



solution and afterward put in a solution of carminic acid, and hence are said to be stained with carmine, but the order may be reversed and the alum used last, and then that is the staining reagent. It is also clear that the tissue does not always take the same color as the staining liquid, as for example, a solution of white indigo will stain wood fibers indigo blue on contact with the air, or a solution of hæmatein, which is brown, will give a violet stain.

LOGWOOD is a colorless preparation when fresh, but fermented or ripened it becomes dark red-brown, or carmine-red since the hæmatoxylin present has been in part changed to hæmatein. This was used at one time quite extensively on account of its cheapness, but the tannin of the wood is unfavorable to good results.

*Hæmatoxylin* ( $C_{16}H_{14}O_6$ ) is the coloring principle of logwood, and is extracted by the use of ether containing some amount of water. The crystals when fresh are colorless, but owing to ready oxidation soon become violet tinted. Solutions oxidize in the presence of alkalies and pass into brown hæmatein or over into higher oxidation products. It contains a weak acid and forms salts, which take up acids readily and become hæmateates. To produce a stain, hæmatoxylin alone is good for nothing; a base must be present with it, in one of the following ways: in objects already prepared; in a mordant used before or afterwards; in the tissue at the same time as the stain. As bases those mostly used are aluminum, chromium, iron, and copper, and the salts which rise from these; hæmatoxylin never contains these.

*Hæmatoxylin Alone.*—To stain plant cells, tissue is first fixed in picric acid and well washed, some stain is left on the slide, and ammonia fumes poured over. Ammonium-hæmatein is formed and the nuclei, especially, stained violet. Just what base comes into this reaction is unknown, probably iron and copper. Animal tissues stain but little in this solution. What takes place in using this stain depends entirely upon the mixture or methods used.

*Hæmatoxylin with Chromium.*—Objects were first treated with potassium-bichromate and later with the monochromate, since this holds in the tissues better. After treatment for from twelve to fourteen hours in a one-third per cent. aqueous solution of hæmatoxylin, the sections were left equally long in a one-half per cent. solution of potassium monochromate, and then passed up through alcohol and xylol into paraffin. Weigert's modification is especially for the sheaths of the central nervous system. Material is hardened in Müller's or Erlicki's fluids, and sections, before the tissue has become green, are put from one to two hours at 35° to 40° degrees C. in a three-fourths per cent. of ten per cent. alcoholic hæmatoxylin, which stain must be several days old and contain hæmatein. Then after the sections have become black, they are put into a mixture of borax (four gr.); red potassium ferrocyanide (five gr.), and water (200 cc.), and left till the gray matter is yellow. A modification is made by treating the sections with one-half per cent. chromic acid for a few minutes before putting them in the stain. Other modifications differ only in treating the overstained sections with potassium hyper-manganate (one-fourth per cent. solution), and oxalic acid and potassium sulphite (1-200 water). This leaves the gray substance colorless, and the white blue, and the sections may be further stained with carmine.

*Hæmatoxylin and Iron.*—As with copper and chromium, the iron is in chem-

ical union with the hæmatoxylin, and there is a higher oxidation product present than hæmatein. Usually after treatment with iron the sections are put in a hæmatoxylin solution till diffusely colored and then differentiated by an acid mixture either in the iron mordant or else free. Many modifications of this method are too well known to need description; Heidenhain's the best perhaps. A special process of his is to previously stain in Bordeaux red or anilin blue, to prevent the iron from going into chromatin and plasma. To stain the cell plasma, Butschli brings the sections first into a weak solution of iron acetate, then into the hæmatoxylin, and does not differentiate further.

*Hæmatoxylin and Copper.*—The principle of this combination is the same as with the preceding metal; a copper salt was used before in combination with or after hæmatoxylin; copper sulphate and copper acetate are both used, and in a certain form this process constitutes the famous Weigert method. Weigert hardens tissue in potassium bichromate, imbeds in collodion, and treats for two to three days in a half saturate solution of copper acetate, following this with a weak alkaline hæmatoxylin ( $\frac{3}{4}$  to 1 gr. hæm., 10 gr. alcohol, 90 gr. water, 1 gr. concentrated solution of lithium carbonate) containing lithium carbonate, which aids in the formation of hæmatein. He finally differentiates with his potassium ferrocyanide solution weakened one-half. Many modifications of this method have been made, but none have changed the principle of reactions. Weigert himself uses as a mordant equal volumes of a concentrated solution of copper acetate and a ten per cent. solution of potassium sodium tartrate, but treats again with copper acetate before sectioning; the hæmatoxylin is of a somewhat different composition, and weak acetic acid is used in differentiation.

*Hæmatoxylin and Molybdenum.*—This is used for nerve tissue by several workers, and most simply by staining the tissues in an old mixture of hæmatoxylin and ten per cent. phospho-molybdic acid (1 pt.), chloral hydrate (6 to 10 pts.), and water (100 pts.).

*Hæmatoxylin and Vanadium, etc.*—Objects are fixed in potassium bichromate and copper sulphate in fifty per cent. alcohol, cut in collodion, and the sections mordanted in a mixture of one part of ten per cent. solution of vanadium chloride and four parts of eight per cent. aluminum acetate, treated with a copper hæmatoxylin, and differentiated in hydrochloric acid alcohol. Axis cylinders, ganglion cells, etc., are stained, and sheaths not. Which of the four metals, aluminum, chromium, copper, or vanadium, plays the most important part, is not known.

*Hæmatoxylin with Other Metals.*—Heidenhain found magnesium, strontium, etc., useless, other authors believed themselves to have demonstrated the presence of calcium carbonate in egg yolk by the use of hæmatoxylin; results are not yet final. Hermann uses an alcoholic hæmatoxylin and differentiates by Pal's modification of Weigert's method, using sodium hypermanganate in the mordant, but since the tissue is hardened in platinum chloride and osmic acid, the metal acting is not clearly known. Other metals have been used, zinc, bismuth, cobalt, nickel, tin, uranium, wolfram, but are not in common use.

**HÆMATEIN.**—This can be used alone as a stain for the cell nucleus, mucous substances, and nerve fibrils; according to Mayer this is due to the union of

nucleic acid with aluminum from the alum, and hæmalum from the aluminum salt of hæmatein.

*Hæmatein with Aluminum.*—This gives pure nuclear stains only when alum is relatively abundant, or when aluminum chloride is in excess in an alcoholic soluble chloride ( $\text{CaCl}_2$  preferably). The presence of free acid or alum is desirable for mucin, or, better, a mixture containing relatively much hæmatein diluted with potassium acetate, spring water, etc.

*ALUM-HÆMATOXYLIN.*—Of the many preparations of this form of stain the best are: *Delafield's*, which is formed of a saturated solution of ammonium alum (100cc.), with a solution of one gr. of hæmatoxylin in six cc. of strong alcohol. This mixture is allowed to stand three to four days in an open flask, and 25 cc. of glycerine and methyl alcohol are added, and the whole allowed to stand at least two months in the open until it is dark colored. *Erlich's* mixture is composed of one gr. hæmatoxylin, fifty cc. of absolute alcohol, five cc. of acetic acid, fifty cc. of glycerine, and water and alum in excess. This is allowed to become dark red in an open flask, and if well corked keeps for a year or more. Sections stain very rapidly, and masses should be stained through but not over-stained; sharp nuclear stains result, owing to the acid present in the older mixtures.

Several other hæmatoxylin mixtures are taken up that must be passed over, and these stains left with this incomplete review. The group of cochineal stains is next considered.

*COCHINEALS* are obtained from the female cochineal insect *Coccus cacti*, by grinding and extraction.

*Alum Cochineal.*—Into a five per cent. solution of alum is put finely ground cochineal. This is boiled, filtered, and some salicylic acid added for keeping it. Another process (Crooker's), is to take cochineal alum (7 g.), and water (700 cc.). This is reduced to 400 cc. by boiling.

*A New Cochineal Tincture.*—(Mayer.) This is made of cochineal 10 gr., calcium chloride 10 gr., aluminum chloride 1 gr., nitric acid 16 drops, alcohol (50 per cent.) 200 cc. The finely pulverized cochineal and salts are mixed with the alcohol, and acid added, and the whole heated to boiling point; this is allowed to stand cold, but well shaken for a few days, then filtered.

*CARMINE.*—This is a combination of carminic acid with aluminum and calcium, and a not well known albuminous substance, which is obtained from cochineal. It is easily soluble in caustic alkalies, carbonates, borax, and acidulated alcohols.

*Acid Aqueous Mixtures.*—Alum-carminic (Grenacher). An aqueous solution of one to five per cent. potassium or ammonium alum is boiled for ten to twenty minutes with one-half to one per cent. carmine, and filtered after cooling. This is however, a stain of poor penetration, but does not over-stain. Acetic acid carmine is made by boiling a forty-five per cent. solution of acetic acid till saturated with carmine. This is used as a stain for living objects, and gives a sharp, nuclear stain, but is not permanent. Formic acid carmine is also used, and one author puts his objects stained in acetic acid carmine into a one per cent. solution of iron oxide-ammonium-citrate, forming as a stain iron carminate.

*BASIC AND SO-CALLED NEUTRAL AQUEOUS MIXTURES.*—Magnesium, lithium,



borax, and ammonium carmines come under this head, and are prepared by boiling carmine in certain per cent. solutions of the basic or neutral substances. These are not very vigorous stains and those strongly alkaline are liable to macerate the tissue.

**ALCOHOLIC MIXTURES.**—Ordinary borax carmine (Grenacher's), which has been reduced by boiling, is completed in volume by a seventy per cent. alcohol. This makes a stain of good penetration and avoids maceration or treatment with aqueous solutions. It can also be washed out with acid alcohol and left as a pure nuclear stain. Hydrochloric acid carmine (4 g. carmine, 15 cc. of water, 30 drops HCl.) is boiled to concentrate, and 95 cc. of eighty-five per cent. alcohol is added. This is filtered hot, neutralized with ammonia till a precipitate appears, and finally filtered. Chloral carmine is made by adding a few grains of chloral hydrate.

**MIXTURES FOR DOUBLE STAINING.**—Picric acid or some of its salts are chiefly used for double staining, either with or after the other stain. The well known picro-carmine is a most variable mixture of picric acid, ammonia, or sodium, or lithium, carbonic acid, also sometime acetic acid and carmine. Picro-carmine contains only a little free ammonia, and enough ammonium carmine to give a good double stain. The result is just as well or better gained by first staining with borax or para-carmine, and afterwards with picric acid in alcohol or benzol, etc. Indigo-carmine and carmalum or hæmalum give a blue stain to plasma in balsam, after borax-carmine, which gives the nuclear stain. Among other counter stains, Lyons blue can be most successfully used; it brings out differentially cytoplasmic structures, as the cell body and formative yolk in developing eggs.

A. M. C.

**Eternod, Dr. Prof., A. C. F.** Instruments et procédés micrographiques nouveaux. Zeit. f. wiss. Mikr. 15: 417-427, 6 figs. 1899.

This describes a new mechanical stage, an adaptation of Greenough's binocular for use on an ordinary microscope, apparatus for squaring paraffin blocks, and a model for mounting sections.

The instrument for squaring paraffin blocks to be cut in serial sections, or for reconstructions, is shown in Fig. 1. Its principal parts are a vertically-moving knife-carrier (A), a knife (B), a carrier (C), moved horizontally by a special adjustment screw (D), and surmounted by a support for the paraffin holder, and a plate mounted upon a graduated disc (F) with a pivot, for measuring the angle

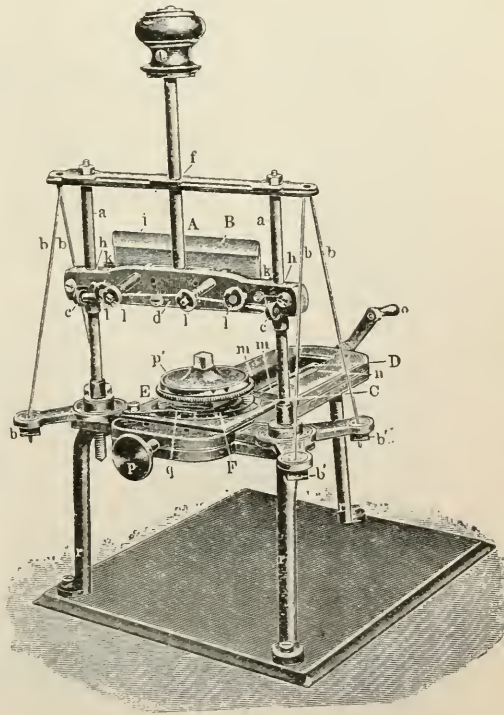


FIG. 1



at which the cutting is to be done. The knife-carrier (A) moves on the upright steel rods (a, a,) kept rigid by the braces (b, b, b, b,) each of which may be regulated with a bolt. Even motion is further assured by two rollers (c, c,) pressed against the steel bars by a strong spring to which they are attached, and also by passing the handle of the knife-carrier through the cross bar at (f) giving a third support at a higher level than (h, h,) where the upright bars pass through the carrier.

The knife (Fig. 1 B and Fig. 2) is a thick steel blade with two beveled edges

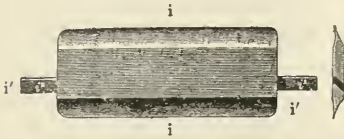


FIG. 2.

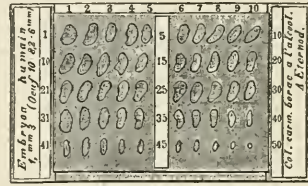


FIG. 3.

(i, i,) and two handles (i', i') which fit in the oblique grooves (k, k,). The knife is kept firm at the required angle by four paired screws (l, l, l, l,). The horizontal carrier (C) has two lateral grooves by which it rests on the horizontal slide (m, m,) which has a scale (n), for determining the exact size of the paraffin blocks. The adjustment screw (D) moved by the lever (o) turns the carrier with the paraffin block in the horizontal plane. This screw has a pitch of about  $\frac{1}{2}$  mm. and the end of it is hollowed out to fit the point of the set screw (p) which holds it in place.

At the base of the support (E) for the paraffin holders which go with the microtome, there is a graduated disc (F) with regular notches to receive the spring click (q). By means of these the paraffin block can be accurately oriented and cut in any form desired.

To manipulate the instrument, the knife is fixed at the desired angle, the knife-carrier raised, the paraffin block oriented and bits of it chipped off, the carrier being turned by the lever (o) after each stroke until a first surface is defined, then the graduated disc is turned at the desired angle and the other surfaces are cut.

With the apparatus, blocks may be cut varying in size from less than 1 mm. to those 7 cm. high and with a base 8 cm. square. If desired, a notched knife for tracing lines may be used instead of the razor. The parts of the instrument are made of bronze, brass, nickel and steel. The different pieces may be detached and readjusted by screws. When the knife carrier is raised, the microtome is about 35 cm. high, the horizontal slide gives the carrier a play of 8.5 cm. and the knife may be moved through 7 cm. The instrument rests on three legs screwed to a firm brass plate 20 cm. long, and 18 cm. wide.

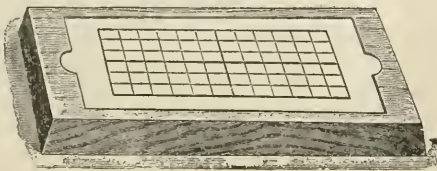


FIG. 4.

The model for mounting sections (Fig. 4) is a block of wood having a depression the size of the slide,

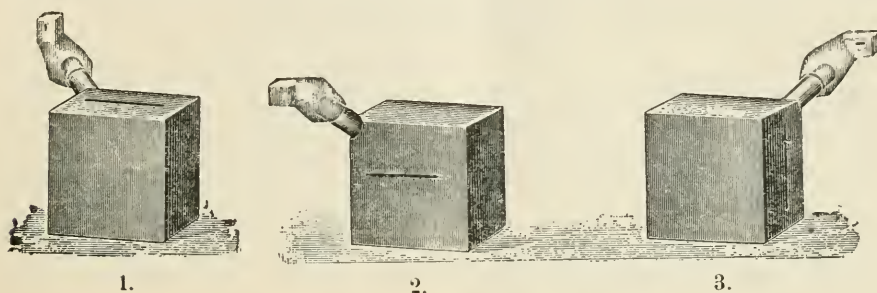
and marked with lines to show the exact position for the sections. It is a good plan to number the rows of sections on strips of paper gummed to the slide and to the cover (Fig. 3). This will show any error in the mounting of the series, and the strips of paper protect the glass which may be easily broken after one has worked several months on the series.

E. M. BRACE.

**Noack, Wilhelm,** Eine Methode zur Orientierung kleiner Objecte beim Zerlegen in Schnitte. Zeits. f. wiss. Mikr. 15: 438-443, 1899.

With this method small objects may be accurately oriented by the use of the microscope, and both cross and longitudinal sections may be made from the

same specimen. A metallic cube measuring 16 mm. on each edge has a bar with rough upper surface for the paraffin block, fitted on diagonally at one corner (Fig. 1). A line drawn on one side of the cube serves to mark that surface in different posi-



tions. The paraffin block may be cut in three different planes, making cross, frontal and sagittal sections—as may be seen by turning the marked surface of the cube about (Figs. 1, 2, 3). The cube, carrying the paraffin block is clamped in position in the microtome, and a line made on it along the edge of the plate that holds it in place so that the cube can be removed and brought back to the same position again. A trial section is now cut, and placed under the microscope and the cube with the paraffin block placed close to the side of the

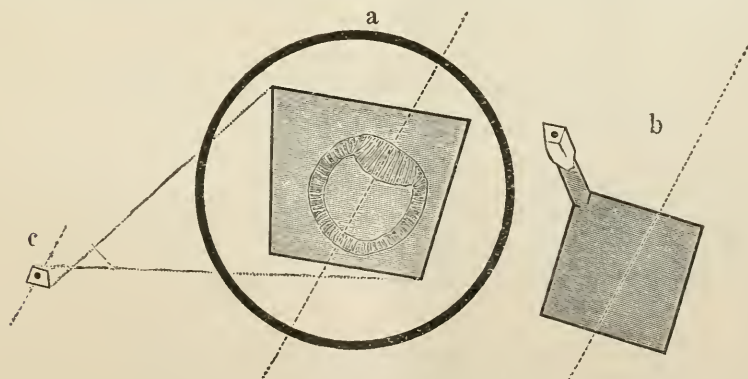


FIG. 4.

microscope, in a corresponding position. By looking through the microscope at the section with one eye, and at the cube with the other, the dorso-ventral plane of the object may be determined, and a line drawn on the cube to indicate the direction in which it lies. (Fig. 4.) The fact that the microscopical image is

turned through 180 degrees does not affect this relation. If necessary, the position of the paraffin block on the bar may be changed. The cube may be brought back to its original position in the microtome by means of the line drawn at the edge of the clamp, and the sections cut. Different parts of the object may be cut in different planes without waste of material since its position is definitely known.

E. M. BRACE.

## CURRENT BACTERIOLOGICAL LITERATURE.

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Separates of papers and books on bacteriology should be sent for review  
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**A Manual of Bacteriology.** Herbert U. Williams, M. D., Professor of Pathology and Bacteriology, Medical Department, University of Buffalo. Pp. X., 263, 78 illustrations. P. Blakiston's Sons & Co., Phila., 1898.

Dr. Williams' manual is not intended to be an exhaustive treatise on bacteriology, nor to take the place of the larger text-books, but rather to present in concise and intelligible form the

more important facts of the science and its special technique, for the benefit of beginners and of physicians who have not had an opportunity to obtain much laboratory training in this subject.

The book is divided into four parts, dealing respectively with bacteriological technique, the classification and biological characteristics of bacteria (including a discussion of toxines, immunity, etc.), non-pathogenic, and pathogenic bacteria. A chapter on disinfectants and antiseptics is contributed by Dr. Thomas B. Carpenter, and one on preparation of instruments, ligatures, dressings, etc., for surgical purposes, by Dr. Chauncey P. Smith.

The topics discussed in the book are viewed from the medical standpoint distinctively, and the facts which most concern the physician are discussed in such a plain and thoroughly practical manner as to be readily understood even by one who is not at all familiar with the subject from its laboratory side.

It is to be hoped that the manual may have a large circulation, even if for no other reason than to correct the pronunciation prevalent among medical men of the generic terms ending in "cocci," as micrococci, staphylococci, and streptococci. The physician who fails to pronounce these words as though they ended in "cock-eye" is very seldom met. For the proper pronunciation see Dr. Williams' manual, page 11, or any dictionary.

CHARLES WRIGHT DODGE.

**Coplin, W. M. L., M. D.** The Propagation of Diseases by Means of Insects, with Special Consideration of the Common Domestic Types. Phil. Med. Jour., pp. 1-15, June 10, 1899.

Insects were brought in contact with cultures of pathogenic organisms, and afterwards allowed to walk over set agar Petri plates. Cockroaches, bed-

bugs, and flies were used in the experiments. Cultures of the pathogenic forms were obtained in this way, but not pure cultures, as each insect carried a load of

extraneous organisms, among which the pyogenic bacteria predominated. It appears that certain insects perpetuate or actually spread diseases, and that they may be potent agents in the transportation of bacteria for twenty-four or forty-eight hours, or perhaps longer, after infection. E. M. BRACE.

**Graham, J. S.** The Preservation of Culture Media. *Ga. Jour. Med. Surg.*, March, 1899. As a method for effectively sealing tubes of culture media, the writer recommends depressing the cotton plug and dipping the mouth of the tube into boiling white beeswax after sterilization. After cooling slightly, the tubes are again dipped in wax that has been cooled until it is beginning to solidify.

E. M. BRACE.

**Staining Method for Blood Preparations.** This is a method for bringing out the nuclei, the ætrophilous, eosinophilous, and basophilous granulations, and the blood plates. The preparation is fixed by the Ehrlich method, or by treating with alcohol from one-half to twenty-four hours. Solution A consists of twenty parts of methylen blue with twenty parts of absolute alcohol. Solution B consists of twelve parts of eosin with 28.9 parts of acetone. Pour together one cc. of each of these and dip the preparation into the mixture. The preparation turns blue, then red, and should be removed from the stain the moment the red reaction appears. The time required varies from one-half minute to ten minutes. E. M. BRACE.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Harvard Medical School, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

**Labbé.** Le ganglion lymphatique dans les infections aiguës. *La Presse Medicale.* 4 plates, March 22, 1899. The lymph nodes from cases of erysipelas, typhoid fever, diphtheria, lobar pneumonia, and broncho-pneumonia were studied. In erysipelas, in the adult, the lymph nodes near the inflammation were found to be congested. The sinuses contained desquamated cells, often showing evidence of phagocytosis. The follicles were little altered. In the new-born, however, the lymph node was hæmorrhagic, œdematous, the follicles necrotic, and the lymph sinuses obliterated by phagocytic cells and altered lymphocytes and polymorphonuclear leucocytes. In the walls of blood vessels inflammatory changes were seen. In the adult the streptococci were seen only in the lymph sinuses; in the new-born they were seen scattered diffusely through all parts of the node.

In typhoid fever the mesenteric lymph nodes only were examined. During the first week of the disease hyperæmia was the only change noticed. At the height of the disease, the follicles contained lymphocytes with altered, shrunken nuclei. Often the centers of follicles were necrotic. The lymph sinuses were dilated and contained many large pale cells with vesicular nuclei. These cells were markedly phagocytic. Some of the lymph nodes nearest the



intestine were entirely necrotic. After defervescence there may be almost complete reparation with only a little connective tissue formation around the blood vessels. Abscess of the lymph node or complete sclerosis may occur.

In acute lobar pneumonia the tracheal lymph nodes were congested and often hæmorrhagic. The centers of the follicles were invaded by polymorphonuclear leucocytes, which appear early and are afterward destroyed by the cells of the reticulum, which multiply rapidly and are markedly phagocytic. These cells in turn undergo a granular degeneration, and are carried off by the lymphatics.

In acute broncho-pneumonia the changes are similar.

In diphtheria the cervical lymph nodes were examined. These were congested and often contained abscesses due to secondary infection. In some nodes was seen fragmentation of nuclei in the center of the follicles, with occasional foci of necrosis.

R. M. P.

**Labbé.** Les ganglions lymphatiques dans les processus infectieux. *La Presse Médicale*. April 12, 1899.

In this article Labbé reviews his previous work and draws the following conclusions in regard to the rôle

of the lymph nodes in infectious processes.

By its system of lymph sinuses the lymph node arrests the onward progress of bacteria which have escaped the action of the leucocytes at the point of inoculation, and of the endothelial cells of the lymph vessels through which they have passed.

In the lymph node the bacteria are subject to a second phagocytic action, that of the endothelial cells of the lymph sinuses. These cells also aid in removing the excess of red blood globules and degenerated cells.

Bacteria do not invade the lymphoid tissue unless the resistance of the tissue has been diminished by an earlier action of toxin absorbed from the site of inoculation.

The lymph node not only retards the progress of bacteria, but also attenuates their virulence.

During infection the production of leucocytes by the follicles of the lymph node is increased.

These changes take place in both local and general infections.

R. M. P.

**Bezancon et Labbé.** Sur la réaction des ganglions lymphatiques au voisinage des cancers. *La Presse Médicale*, April 15, 1899.

Lymph nodes in the vicinity of carcinoma are often hypertrophied without any invasion by the growth. His-

tologically the follicles are very large, their germinative centers are highly developed and rich in karyokinetic figures.

R. M. P.

**Engelken.** Metastasierende embryonale Drüsen-  
geschwulst der Nierengegend im Kindes-  
salter. *Beiträge zur Path. Anat. und Allg. Path.* 26: 320, 1899.

The writer reports a new growth in the kidney of a girl four years old, which by direct extension involved the vena cava and the right auricle of the

heart. There were many metastases in the liver and the peritoneum, and one nodule in the left lung. Microscopically the new growth consisted of sarcoma-like cells, connective tissue, blood vessels, smooth muscle fibers, and gland-like

structures with alveolar formation, as well as actual lumina lined by one or more layers of cuboidal or cylindrical cells. Metastases showed a similar appearance.

Twenty-eight cases of similar embryonal tumors were collected, tabulated, and divided into three groups: first, those with sarcomatous structure, epithelial gland-like structures, and smooth muscle fibers; second, those similar to the above but with striated muscle; and third, those containing also other tissues as cartilage. In most cases the kidney structure was not preserved, hence the writer concludes that the embryonic *anlage* of the kidney was involved as well as the Wolffian bodies.

A. M.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace,  
131 Park Avenue, Rochester, N. Y.

**Langley, J. N., F. R. S.,** President of the Physiological Section of the British Association.  
Opening Address. *Nature*, **60**: 557-562,  
1899.

The address presents some general conclusions regarding certain problems in neurology. Stress is laid upon the point that in any broad classification,

not more than two tissues—striated and unstriated muscular tissue—are known to be supplied with approximate completeness with efferent nerve fibers, and that we have immediate control over but one tissue—the fibrous striated muscle tissue. The nervous system has limited control over cellular activities. It may affect the secretion of a gland, but as far as we know, it does not affect such life processes of the gland as the taking up of oxygen and the giving out of carbonic acid, the essential effect of a nerve impulse appearing to be the modification of the amount of energy set free as work.

The fibrous striated muscle tissue is the only one over which we have immediate control, and the tissue, with the nervous system supplying it, may be placed in a separate class as somatic or voluntary tissue.

It seems probable that in the evolution of mammals the sympathetic nerves developed at one time, and the cranial and sacral involuntary nerves at another. This hypothesis is based upon the fact that nerves from the two regions usually produce different effects, while fibers passing by spinal nerves to any one part of the involuntary system produce effects that may vary in degree, but not in kind, in that part. With regard to inhibitory fibers, their development cannot be looked upon as universal throughout the organism any more than the development of motor fibers. There is not sufficient evidence for the theory of an equal endowment of tissues with nerve fibers.

With regard to specific nerve energy, it seems certain that the different classes of nerve cells and fibers do not have the inherent differences required by this theory, although there may be some comparatively superficial differences to explain the selective power observed.

Evolution in general is determined by the same factors that determine the supply of nerve-endings in a tissue and the degree of their development.

E. M. B.

**Eigenmann, C. H.** The Eye and Hearing of the Blind Fishes. *Proc. Ind. Acad. Sc.*, 1898. pp. 242-243; 2 pls.

Prof. Eigenmann disputes the statement made by Cope with regard to Amblyopsis, that the presence of an enemy can be discerned only through the medium of hearing. He has tried whistles, tuning forks, clapping of hands, shouting, and found that noises of all kinds were disregarded, although the ear of Amblyopsis is normal in structure.

E. M. B.

**Meyer, Dr. Semi.** Ueber Centrale Neuritenendigungen. *Archiv. f. Mikr. Anat.* 54: 296-311, Tf. 17, 1899.

The writer protests against various views expressed by different investigators concerning the nature and function of the pericellular network. He considers that it is the central termination of neurites, which, coming in this way in the closest contact with the surface of the cells they surround, facilitate the reception of stimuli by the cell.

Evidence against the theory suggested by Golgi, that it is an insulating medium, is found in preparations in which the neurite may be traced directly into the network. That the appearance is not due to the reticular structure of the protoplasm is shown by sections of nerve cells in which the network does not penetrate, but may be clearly seen to stop on the surface. Material was prepared after Bethe's methylen blue method, so that none of the appearances observed could be due to precipitate of metal, as some students contend.

E. M. B.

**Bethe, Albrecht.** Die Locomotion des Haifisches (Scyllium) und ihre Beziehungen zu den einzelnen Gehirnthellen und zum Labyrinth. *Archiv f. Physiol.* 76: 470-493, 2 text figs., 1899.

Motion was not affected by removal of portions from one side or from both sides of the cerebrum. Removal of the 'tween-brain increased spontaneous motion, but aside from this restlessness

no change was observed. Removal of a portion of the cortex from the posterior side of the corpora bigemina through the base of the mid-brain causes the fish to swim toward the side opposite that operated upon, and in time this is followed by the bending of the body in the same way, until finally the head may touch the tail or lie under it. When the mid-brain is entirely removed, the fish moves toward the left or the right, or in some cases swims on its back, although it will finally turn over. The statement of Steiner that spontaneous motion is lost after the operation, was not sustained by the experiment.

The medulla was removed from several specimens, and in every case the fish swam with rhythmical motions, although somewhat inclined to reel. Stimulation of these specimens stopped swimming motions except when the medulla had been cut between the auditory and the glossopharyngeal nerve.

Correlated motion is undoubtedly influenced by the labyrinth. Longitudinal division of the medulla affects the geotropism of the fish, and this division plus hemi-section in the posterior part produces effects similar to the sectioning of the auditory nerve, but the effects are less pronounced. Cutting the auditory nerve on one side has but slight effect if the medulla is partly cut through in the

indifferent zone. Observations support the view that there is a mingling in the medulla of tracts of the auditory nerve extending caudad into the spinal cord, so that each auditory nerve has fibers from both sides.

From combined operations it appears that the cerebellum affects correlated motion, but this is not apparent when the cerebellum alone is removed. Five specimens in which the cerebellum was extirpated, and one or both auditories cut, or the medulla partly cut across back of the Vagus, held their pectoral fins, under strong tonus, in a position perpendicular to the axis of the body.

E. M. B.

#### RECENT LITERATURE.

- Kirchoff.** Recent Views as to the Topical Basis of Mental Disorders. *Am. Jour. Insan.* **55**: 481-495, 1898-9.
- Havet, J.** L'état moniliforme des neurones chez les Invertébrés. *La Cellule*, **16**: fasc. 1, 1899.
- Weidenreich, F.** Zur anatomie der centralen Kleinhirne der Säuger. *Zeits. f. Morph. u. Anthropol.*, **1**: heft 2, 1899.
- Schultze, L. S.** Die Regeneration des Ganglions von *Ciona intestinalis* L. u. über das Verhältniss der Regeneration u. Knospung zur Keimbälterlehre. *Jen. Zeits. f. Naturwiss.* **33** (N. F. **26**): heft 2, 1899.
- Wundt, W.** Zur Theorie des Gefühls. *Phil. Studien*, **15**: heft 2, 1899.
- Poirier, P. et Charpy, A.** *Traité d'Anatomie humaine*. Vol. III Systeme nerveux, Fascicle 3 (fin): Les nerfs. pp. 747-1233, 205 figs., Paris, 1899.
- Burvlief, J. van.** Noyau d'origine der nerf. oculo-moteur commun du Lapin. Limites, structure et localisations.
- Brandes, G.** Die Leuchtorgane der Tiefseefische *Argyrolepecus* u. *Chauliodus*. *Zeits. f. Naturwiss.* **71**: heft 6, 1899.
- Fritz, F.** Ueber die Structur des Chiasma nervorum opticorum bei Amphibien. *Jen. Zeits. f. Naturwiss.* **33** (N. F. **26**): heft 2, 1899.

## NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA MCI. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

**Gramont, A. de.** Analyse spectral des minéraux non conducteurs, par les sels fondus. *Bull. Soc. Min.* **21**: 94-130, 1898.

A very exhaustive treatise on the subject. The spectrum obtained by the action of an electric spark upon the melted salt, supported on the flattened end of a thick platinum wire. This method generally gives better diagnostic results than with either blowpipe or wet analysis. Two to three parts salt used to one of the mineral. Carbonate of lithium used as flux, lithium giving a very simple spectrum and having other advantages. Apparatus for producing spark, and spectroscope, described in detail. Spectra of the different elements and common minerals given. Most of the simple bodies give satisfactory results, but these are, however, defective or insufficient for the iron minerals, fluorite and boron. In cases of mixtures of many elements the "condensation" can be diminished, thereby getting only the spectra of the metals.

L. MCI. L.



**Joly.** On the Change in Volume of Minerals near their Fusion Point. Trans. R. Dubl. Soc. 6: 283, 1897.

Author calculates and records the coefficient of expansion of diamond, augite and orthoclase at very high temperatures. The method is to photograph the sphere of the mineral at the different temperatures, these photographs being then very much enlarged and therefrom the coefficient of expansion calculated.

### INDIVIDUAL SPECIES.

**Calcite**, with organic coloring matter. J. Fromme. Jahresber. ver. Naturwiss. Braunschweig. 10: 104, 1897.

The calcite examined was translucent, chestnut-brown in color and occurred in weathered gabbro in the Hartz.

Iron and manganese were absent. Coloring due to 0.2310 per cent. apocrenic acid and 0.0135 per cent. of another organic acid, which must exist in combination as the calcium salts. Carbonated rain water takes up ammonia and humic acids from the soil, and acting on the lime-feldspars of the gabbro would produce the colored calcites.

**Calomel.** Sur les propriétés optiques du H. Dufet. Bull. Soc. Min. 21: 90, 1898.

Strong double refraction established by measurements made on convenient artificial crystals.  $n_{\epsilon} = 2.6559$ ,  $n_{\omega} = 1.97325$ .  $(n_{\epsilon} - n_{\omega}) = 0.68268$  (Na light). Dispersion very great, especially for the extraordinary ray. In convergent light the rings of the interference figure appear a little broken, but probably not on account of optical anomalies.

L. McL. L.

**Mica Group**, The Crystal Symmetry of the Minerals of the. T. L. Walker. Am. Jour. Sci., iv., 7: 199, 1899.

Author comments on very general tendency to degradation from higher to lower symmetry, shown by physical and morphological examination of minerals. *Phlogopite* and *biotite*, in very thin plates, proved to be triclinic and not monoclinic by marked divergence of axial plane from parallelism with ray of percussion figure, and by asymmetric etch figures of Wiik on basal cleavages. Thicker basal plates do not show this divergence, probably due to polysynthetic twinning, with (001) as composition face. *Lithia micas* appear monoclinic optically, but still show triclinic etched figures (by Baumhauer and Wiik). *Muscovite* shown to be monoclinic optically and by etched figures (by Leydolt, Baumhauer, and Wiik). Paper emphasizes the conclusions of Wiik.

L. McL. L.

**Periclase.** Production artificielle de la, par un nouveau procédé. A. de Schulten. Bull. Soc. Min. 21: 87, 1898.

Caustic potash melted with magnesia in a silver capsule to a red heat, cooled slowly, and after washing with chlorine water, crystals obtained and subsequently purified. Analysis shows 99.92 MgO, with trace of Fe.  $G. = 3.568$  at  $15^{\circ}\text{C}$ .

L. McL. L.

**Powellite Crystals** from Michigan. C. Palache. Am. Jour. Sci., iv, 7: 367, 1899.

Two crystals ( $\frac{1}{2}$  to 1 cm. in height) obtained, pale bluish-green in color, translucent and with subadamantine lustre.  $G. = 4.353 - 8$ . Measurements made on two-circle goniometer (Goldschmidt model) and angles recorded in  $\varphi$  and  $\rho$ .

Forms observed: *p* (111), *e* (101), *h* (133), *j* (3.11.11), *k* (155), *l* (1.11.11); with the two latter faces tending to form a curved surface. Pyramidal hemihedrism strongly emphasized, and measurements confirm axial ratio of Melville and establish isomorphism of the species with scheelite group. Author mentions probability of some darker blue, more massive powellite proving to be pure calcium molybdate, thus constituting the missing end of the scheelite-powellite series.

L. McI. L.

**Senaite.** A new Mineral belonging to Ilmenite Group from Brazil. E. Hussak and G. T. Prior. *Min. Mag.*, 12: 30, 1898.

Black in color, with sub-metallic lustre. Streak, brownish-black. Non-magnetic. Transparent in thin splinters.

Composition =  $\text{Ti O}_2$ , 57.21;  $\text{PbO}$ , 10.51;  $\text{FeO}$ , 4.14;  $\text{Fe}_2\text{O}_3$ , 20.22;  $\text{MnO}$ , 7.00;  $\text{MgO}$ , 0.49;  $\text{SnO}_2$ , 0.11 = 99.68. Two analyses recorded, but leading to no satisfactory formula. Approximately (Fe Pb) 0.2 (Ti Mn)  $\text{O}_2$ . Rhombohedral (trigonal rhombohedral of Groth) with  $c = 0.997$ . Rich in faces, and "supplementary" twins very common. Rhombohedral planes bright, while basal planes (due to twinning) and prism faces dull. Weakly doubly refracting, non-pleochroic and uniaxial. No cleavage and conchoidal fracture.  $H. = 6 \pm$ .  $G. = 4.22$  to  $5.301$ . Infusible before blowpipe. Decomposed by boiling sulphuric acid. Found in rounded fragments and rough crystals (which may be partially decomposed) in diamond-bearing sands of Diamantina Minas Geraes.

L. McI. L.

**Sphaerostilbite.** G. T. Prior. *Min. Mag.*, 12: 26, 1898.

Improbable that the mineral exists which was described under this name as a sub-species by Beudant. Heddlé's sphaerostilbite probably consists of thomsonite in sheaf-like aggregates implanted upon more compact thomsonite. The name should be discarded as a structural term applied to stilbite.

L. McI. L.

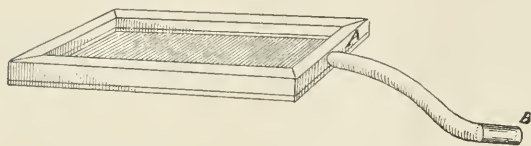
## NEWS AND NOTES.

ANSWER TO CORRESPONDENT.—The cover-glasses that could not be cleaned after treatment with  $\text{HNaCO}_3$  were probably chemically acted upon by the solution. Alkalies have an etching effect upon glass, especially when hot solutions are used.

APPARATUS FOR REMOVING BUBBLES FROM MOUNTS.—In the course of my peregrinations among the laboratories of the North of England, I have, upon several occasions, met with a small exhaust apparatus which is used for the removal of air bubbles from mounts. It is simple in construction, and effective in action, and a description of it may not, therefore, be without some interest to readers of the JOURNAL OF APPLIED MICROSCOPY. It consists of a slip of plate glass measuring  $4'' \times 1\frac{1}{2}''$ , to which a frame of wood has been cemented. The frame is of such a size as to allow of ordinary glass micro-slips being placed in it.

In one side of the frame a hole is bored, and one end of a piece of india-

rubber tube, measuring six inches long by three-sixteenth inch diameter, is cemented into it. A piece of glass tubing (B) one inch in length is closed at one end;



and a small hole is bored through it at about a quarter of an inch from the closed end. The open end of the glass tube is then slipped into the free end of the rubber tube, and the two so arranged that the hole in the former shall be just covered

by the india rubber. To use the apparatus, place the mount containing the air bubbles in the wooden cell, and cover it over with a second slip of glass measuring  $4'' \times 1\frac{1}{2}''$ , the edges of which have been previously greased with tallow. If the frame has been accurately made the cell will now be air tight. Exhaust the cell by drawing the air through the tube. The valve formed by the hole will prevent the re-entrance of air, and any bubbles contained in the mount will quickly disappear.

J. H. COOKE.

London, Eng.

ON THE BACTERIOLOGICAL TESTS APPLIED TO THE EFFLUENT OF THE SOUTH NORWOOD IRRIGATION FARM.—Prior to 1894, it had not occurred to the authorities in charge of the sewage farm at South Norwood, London, to utilize the microscope in their examinations of the effluents from the farm. In that year, however, Dr. J. M. Hobson, M. D., B. Sc., undertook to supplement the usual weekly chemical tests with periodical bacteriological examinations, and these were attended with such interesting and valuable results that they have been conducted regularly, at fixed intervals, down to the present time. His microscopical investigations may be broadly divided into two separate processes. The first is an enumeration of the organisms in measured quantities of screened sewage and of effluent respectively; the second is an isolation of specific organisms in the screened sewage and the effluent. The following is a brief outline of the processes and methods that he adopts in his work.

To enumerate the organisms, a sample is conveyed to the laboratory as soon as possible, and surrounded with ice. A measured quantity is taken and diluted with a measured quantity of sterilized distilled water. This is thoroughly mixed, and a measured quantity of the diluted matter is added to a tube of melted, sterilized nutrient gelatin. This is thoroughly mixed by oscillation and the whole mass is poured out on a Petri plate, and instantly covered up.

When the gelatin has set, the plate is placed in an incubator and kept there at the constant temperature of  $20^{\circ}$  C. for two or three days, for colonies to form.

These colonies appear as little beads in the transparent gelatin. By general agreement each colony counts as one organism, i. e., an organic unit without any reference to specific identity. The counting then begins. A black card, ruled off into small squares of equal area, is placed beneath the plate of colonies; these squares are numbered. The number of colonies in a given square is counted with a low magnifier, and the number is noted on a correspondingly

marked white paper. The total number in the several squares gives the number of organisms in the quantity of sample taken.

The second process for the isolation and separate cultivation of specific organisms is conducted as follows:

Plates, as before, are used, and the gelatin is inoculated with a small quantity of the material. Individual colonies are selected for observation, and secondary inoculations are made from them. Pure cultures are thus obtained, and various media are again inoculated with these. The behavior of the growths on the various media under lower ( $20^{\circ}$  C.) and higher ( $37^{\circ}$  C.) temperature are observed, and microscopical slides are made from them.

Dr. Hobson's results are interesting, and he hopes, when he has obtained a sufficient number of them, to be able to trace the effects of heat and of rainfall upon the bacterial population of the sewers.

J. H. COOKE.

London, Eng.

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METHOD OF TEACHING BOTANY.—While the extensive revision in botanical nomenclature has been going on during the greater part of the last decade, the method of teaching the subject has been rapidly evolving until the real live teacher of the subject wonders, first, how under the old method he ever became what he is, and second, how some who still hold to a method much in vogue over ten years ago, can hope to properly place the subject before the minds of the students who are just starting into the field of botany.

It would not be easy to say just what has brought about this change. It may be that when the State Experiment Stations began to be established, some of the leading instructors in this branch of science in the United States saw that there would be a limited demand for men prepared to fill botanical positions, and that they therefore turned more attention toward fitting men to be practical botanists who could not only display a knowledge of the binomial nomenclature but who could tell about the whole relation of the vegetable kingdom, and who could at least attempt to explain the sources and dispositions of plant food. Whatever may have been the cause or causes, the result is thus far at least most commendable; namely, to give the student a fair idea of the plant in relation to its surroundings, the relation of one organ to another, and some notion of the diseases of plants.

However, this is but a decade in an age of evolution, for more men are coming who will attempt to solve new problems with now unthought of apparatus.

E. E. BOGUE.

Agricultural and Mechanical College, Stillwater, O. T.

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THE U-SHAPED FOOT IS CLUMSY.—In saying anything on this subject, I shall most likely be accused of threshing over old straw, but I fancy there is a very large number of persons who use microscopes that will agree with the statements I am about to make. As I have done, so they have done, and may do again and again, viz., purchase a new microscope or a dozen of them, all having the narrow and clumsy horseshoe foot, because it happens to be the style of support for the instrument we select. Nineteen years ago I selected



one or more instruments of some fifteen kinds of stands, that my students and visitors, as well as myself, might have a variety for comparison. Since that time other styles have been added to our number. I have been with these

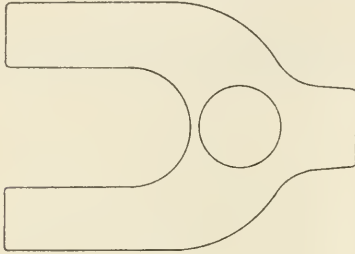


Fig. 1.

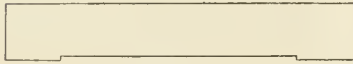


Fig. 2.

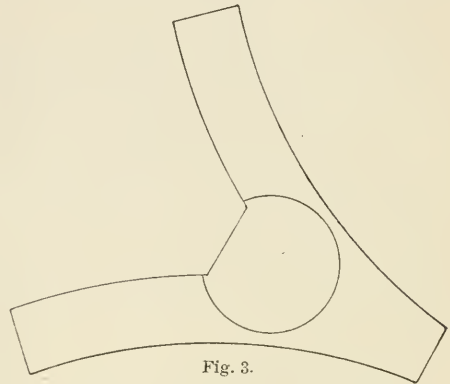


Fig. 3.

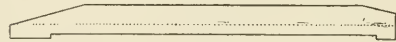


Fig. 4.

instruments in the class-room for many years, and have used all of them myself. I may be prejudiced, very likely, because that it just what I am laying at the door of many others. The manufacturers make microscopes to sell, and in the past, they have made them in wonderful variety, perhaps trying to suit the whim of everybody. Fig. 1 represents the shape of the horseshoe foot; Fig. 2, the same in section as made of solid metal. Fig. 3 represents, on the same scale, the shape of the foot of another instrument used for nearly twenty years past. Fig. 4 shows a section of the same, the dotted lines indicating the depth of the hollow from the lower side. The first instrument mentioned is 30 cm. high and weighs six pounds; the second one is  $28\frac{1}{2}$  cm. high and weighs a trifle over four pounds. If the table on which the first instrument is placed is the least bit curved or uneven, the foot stands on three out of four points and rocks easily, while number two stands firmly on the table no matter what may be the condition of the surface. When placed side by side near each other, erect or inclined  $20^\circ$  to  $30^\circ$ , and a string is tied to the top joining the two, and the feet crowded apart, the heavier instrument tips over before stirring the other one from its "tracks." True, the narrow base can be pressed into a smaller box than the other, but on the table there is room enough for a broader base. For utility and for beauty, it seems to me the horseshoe foot has scarcely a thing to commend it, when compared with the other here illustrated. A considerable portion of the extra two pounds weight seems to be for the purpose of making the stand firm on its "feet," which it fails to do. In the use of microscopes, it has been my need to frequently lift them from one place to another, sometimes with considerable speed. Perhaps the extra two pounds may have been added partially with the view of strengthening the muscles of the arm of the one who handles the instrument; if so, the extra weight has accomplished something.

# Journal of Applied Microscopy.

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## On Studying Slime Moulds.

### SECOND PAPER.

Having in a previous article discussed the study of Myxomycetes in their earlier phases, we pass now to the collection, transportation, and study of the same organisms in fructification or fruit. In some parts of the United States fruit may be obtained at any season of the year. In most localities, however, the best specimens are obtained between May and October. To secure any complete series the student must be in the field almost daily. The plasmodia of some species pass into fruit with great rapidity, and in many cases the fruit is so delicate that the slightest rainfall, or even a strong wind, is sufficient to ruin the beauty of the sporangia, if not destroy them altogether. The student should go forth provided with a strong knife and pasteboard boxes, large and small, in sufficient abundance. My own students find spool boxes most convenient, and they may generally be obtained at stores for the asking. The substratum must be, of course, taken with the fruiting slime mould, and wherever possible should be cut to fit snugly into some box devoted to its reception. Where this is well done specimens come to the laboratory in all the grace and delicacy of their perfect state. Without such care, satisfactory work can hardly be expected. Of course, where time and circumstances favor, a box of glue may form part of the outfit, and the specimens may be cared for by attaching the blocks on which they rest firmly and permanently to the bottom of the box. But even without glue, the writer has carried delicate material from Oregon to Iowa in perfect safety.

Once the specimens are in the laboratory, their further investigation presents no special difficulty; a few suggestions may, however, not be out of place. In the first place, we may proceed to the preparation of our herbarium material. To this end different methods have been employed by different collectors. In the British Museum and in the Schweinitzian Herbarium in Philadelphia, and elsewhere, specimens were cared for by simply placing the material in folded paper packets, which were then mounted on herbarium sheets. It is needless to say that this is not a good method. The material we deal with is generally extremely fragile, and must be absolutely protected. Much of Schweinitz's material has completely disappeared through lack of proper mounting. The most convenient method of dealing with the problem involves the use of small

covered unit-boxes fitted to covered trays of the size of an ordinary herbarium sheet, and about one inch deep. Our boxes are of such size that sixty-four of them fill a tray. It may be convenient to have some of the boxes of double size, of double length or width. In these covered boxes, small pieces of the slime mould-bearing material may be fastened securely with glue. The material should be so placed in the box that it can be examined with a microscope. To this end the sporangia must be raised as high as possible, just so that the box-lid in closing will not touch them. It is sometimes well to mount some of our material in such a way that the sporangia may, by the microscope, be examined from one side. Such specimens should be attached to the side of the box, projecting inward. Mr. Lister, author of "The Mycetoza," prefers fastening the specimens to pieces of rather stiff paper of such size that with the ends bent up a little they just fit inside the box. It is thought that by this arrangement the specimens may be more easily handled, lifted about to the instrument, etc.

But it is time we should bring our material under the lenses. In case of many species it is desirable to view the sporangia as dark objects, to study the surface, measure the diameter, etc. Under a low power some species are well worth such examination simply for their beauty. No natural objects are finer so examined than some of the *Lamprodermas*, *Diachæas*, or *Badhamias*; all these, aside from elegance of form, exhibit luster or iridescence. But to discover structure we must generally resort to other views, other methods of preparation; indeed, we need all the light obtainable from any or every method. Various forms of the *Stemonitaceæ* are well studied as dry mounts. In no other way can one obtain so good an idea of the structure. A dry mount may be prepared as follows: take a bit of rather stiff Canada balsam the size of a pin-head, and place it in such position on the slide that it will at some point meet the edge of your circular cover-glass when laid on well centered. Now lay a single sporangium, which may be detached under the dissecting microscope, over the center of the slide and cover gently, allowing the edge of the cover to touch the bit of balsam as provided. If skillfully done, the amount of balsam will be sufficient to hold the cover firmly, but not enough to run under or at all interfere with your specimen, which may now be examined with great advantage—every part in natural position and condition. When the balsam hardens, the specimen with proper handling will last indefinitely.

For immediate microscopic study I find nothing better than to wet the sporangium on the slide with a little alcohol, then with a weak solution of potassic hydrate. To the preparation glycerine may be added if desired. This will expand all the structures, and give remarkable clearness. Unfortunately this also softens the delicate structures, so that a slight movement of the cover-glass will destroy the preparation. For permanent mounts nothing is better, in my experience, than glycerine jelly. As a preparation, Häntsch's fluid may be used. Care must be taken, in all cases where potash is used, that the solution be not strong, otherwise the delicate tissues are liable to shriivel or be consumed. In some of the *Hemitrichias* the alkali solutions are apt to produce twisting of the capillitial threads to such an extent as to interfere with satisfactory work. In such cases the capillitium may first be drawn out in water, or water and glycerine,

and the alkaline solution run under the cover-glass later on. For such cases some students have found lactic acid helpful, and some recommend mounting in oil.

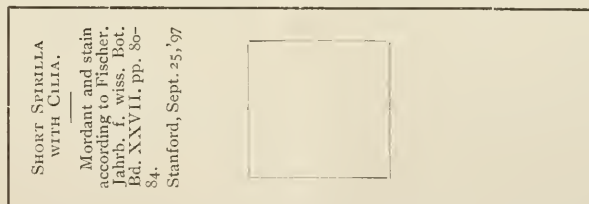
THOMAS H. MACBRIDE.

University of Iowa, Iowa City, Ia.

## Slide-Labeling.

A few years ago I found it desirable to be able to write in ink upon the surface of the slides on which were temporary preparations of the plant parts which I was studying. The oil or grease usually on slides makes writing upon them difficult. Thorough cleaning of the slides with alcohol removes most of the oil, and makes writing with a thick ink and not too fine pen quite possible; but the lines are coarse, and it is impossible to write much on such a slide. One of my friends in Germany suggested painting the end of the dry slide, even without any preliminary washing, unless the slide were stained, with a thin coat of very fluid balsam. Such a coat quickly hardens enough to allow one to write upon it, and by using a drawing pen one can write a great deal on the end of the slide, in fact record there all the stages of the treatment to which the material has been subjected. If it is desirable to make the writing permanent, another coat of balsam after the ink is dry will make it as permanent as the preparation itself. If permanent marking is desirable, it is preferable to use a rather thick ink, Higgins's drawing inks for instance; but a good writing fluid, such as Stafford's, will serve the purpose admirably. In this case the ink must be allowed to oxidize as well as to dry, before being sealed under the second coat of balsam. If only temporary marking is all that is wanted, the anilin inks are preferable, since they can be freely washed off.

I mark all my preparations in this way now, using no gummed labels at all. Slides thus marked can be washed in hot or cold water, with no danger of loosening or removing, or even of obscuring, the label. This advantage is offset, perhaps, by the labels not being as generally legible as white paper ones, but if the slides are held over white paper, the writing at once becomes clear and sharp.



GEORGE J. PEIRCE.

FOWL FLEAS, TO MOUNT.—These show the large muscles of thorax and legs well. Kill and soak for three to six days in ether. Then soaked successively for a day at a time in water, methylated spirit, absolute alcohol, and oil of cloves, and finally mounted in balsam. Mount without pressure by placing three bits of thin glass for the cover to rest on.



## The Bacteria in the Stomach of the Cat.

JULIUS WEISS.

### I.

#### INTRODUCTION.

In his work entitled "Diseases of the Stomach," Hemmeter (1) calls attention to the need of a more thorough knowledge of the bacteria present in the alimentary canal. The serious diseases that are caused in the stomach alone by the action of ordinary invading bacteria, and the close relation that the diseased stomach bears to the heart, blood, lungs, and nervous system, are sufficient reasons to warrant earnest endeavors to elucidate this problem. The fact also that certain species of bacteria, after a sojourn in the stomach for a longer or shorter period, can escape into the intestines, and there, through metabolic activities, set up irritations, or produce toxins that are detrimental to health or even life, most emphatically indicates the necessity of investigating this special department of bacteriology.

Another factor entering here, which must be viewed seriously, is that mentioned by Moore (2), with reference to the Streptococci that may be found on the intestinal mucosa, in larger or smaller numbers, and their capability of penetrating into the various organs of their host, when favorable conditions are present.

Then there is still another point to be considered, which, though it may have for the main part a purely scientific interest, is, nevertheless, very practical. I refer to the probable rôle played by bacteria during digestion. This also is at present an unsolved problem. But, as Professor Howell (3), of Johns Hopkins University, indicates, the knowledge of the digestive processes will not be complete until it is definitely known what part bacteria play in digestion.

Though the subject, in its various phases, has, from time to time, received the attention of a number of investigators, the work accomplished is by no means complete, and a great deal still remains to be done. Investigations, therefore, in which the aims are to help along towards the solution of the problems vaguely indicated above, are at present justifiable and necessary.

Having these considerations in view, I have undertaken a series of experiments at the bacteriological laboratory of the New York State Veterinary College, Cornell University, with the following objects:

I. To isolate the bacteria present on the gastric mucosa of the healthy cat,—during digestion, and after twenty-four hours fasting.

II. To find the variations in the number of bacteria at different acidities during digestion.

III. To determine, as far as time would allow, the rôle that bacteria play during digestion.

From the nature of the investigation recourse had to be had to animal experimentation. In selecting the animal to be used in this research, cognizance was

taken of what is said by Mivart (4), and by Wilder and Gage (5), with reference to the domestic cat,—which animal was used in this investigation.

It will not be necessary here to describe the methods employed, as a discussion of the same is taken up later on in these papers, in proper order.

Each of the three topics of this investigation has a history of its own, which will respectively precede them, reviewing the facts in chronological order.

## I. THE BACTERIA ISOLATED IN THE INVESTIGATION.

The earliest observation on record was that made in 1842, by John Good-sir (6), who described an enormous number of sarcinæ ventriculi which he found in the stomach contents of a young man, suffering from a severe gastric disorder.

Gruby (7), in 1844, in a report to the French Academy of Science, cited a case that came under his observation, of a woman who had suffered for six years from an annoying gastric trouble. A microscopic examination of the stomach contents, revealed the presence of a large number of sporules, some measuring as much as  $4\ \mu$  in diameter, some very much less, and still others considerably more than  $4\ \mu$ , all, however, arranged in beadstrings. From a study of his case, he came to the following conclusions:

1. The parasites present in the stomach did not come from the food digested.
2. They inhabited only the mucosa of the stomach and the œsophagus.
3. They multiplied most actively in the presence of ingested food.

Von Whal (8), in 1861, reported a case of a mycotic affection of the stomach. Embedded in the mucosa of the greater curvature of the fundus, he observed a large number of pustules, which, upon a microscopical examination, appeared to be composed of thickly felted masses of slender filaments. Magnified with a power of 500 diameters, he was able to see very minute spherules, united in the form of beadstrings. From his description, however, it cannot be said with certainty whether or not he dealt with a streptococcic invasion of the mucosa.

Von Recklinghausen (9), in 1864, upon a microscopical examination of a necrotic area of the gastric mucosa, found associated with the lesion a large number of minute sporules. Whether the sporules were micrococci, or merely the spores of a fungus, which was also found present in the diseased tissue, cannot be definitely stated.

Zalensky (10), in the same year, reported a case of hyperacidity in a child two weeks old. Upon *post mortem* examination, the mucosa was found to contain a large number of smallpox-like pustules, which, upon a microscopical examination, revealed the presence of *Oidium albicans*, Robin, and he assigned the affection to be due to this agent.

Klebs (11), in 1881, reported to have found *Bacillus gastricus* in the stomachs of patients suffering with diseases of this organ.

Sternberg (12), in a contribution to the *Johns Hopkins University Studies*, for 1881-3, made the statement that bacteria are present in the alimentary canal of healthy men and animals. He also stated that it is possible for bacteria present in the stomach to escape the action of the gastric juice, and get into the intestines.

Kundrat (13), in 1884, investigated a case in which he found *Favus universalis*.

The parasites had penetrated the mucous membrane of the stomach and intestines, and caused a diphtheritic inflammation of the affected tissues.

Dickson (14), in 1885, reported a case of a dairymaid, who was taken ill with abdominal pain and fever, and who suffered especially from flatulence, eructations, and vomiting. Upon a careful examination of his case, he concluded that the disease was caused by the presence in the stomach of a spore-bearing bacterium, having square cut ends. He was also of the opinion that the organism in question had its normal habitation in the stomach, but that usually it multiplied in insufficient numbers to have any effect upon the peptic glands.

Nasse (15), in 1886, described a case of a mycotic affection of the stomach in a patient 60 years old. Upon *post mortem* examination, the affected area of the mucosa was found to be invaded by numerous rod-shaped organisms, with round ends. From his brief description, however, it is impossible to state what organisms he studied.

A. de Bary (16), in his lectures, published in 1887, mentioned that *Bacillus amylobacter* is constantly present in the stomachs of ruminants.

Richter (17), in the same year, had charge of a patient, a man 64 years old, who suffered from pyloric constriction. An examination of the stomach contents, from time to time, showed the presence of a large number of *Sarcinæ ventriculi*. *Torulæ* were associated with the *Sarcinæ* and in equally large numbers.

Van Puteren (18), in 1888, reported to have isolated a number of micro-organisms from the stomach contents of infants, varying in ages from three to seventy days. All the necessary care was taken of the children during the entire period of the experimentation, and only healthy subjects were employed. Some of them were fed with human milk, while others with cows' milk. Parts of the stomach contents were then removed with a sterile stomach tube, commencing immediately after feeding, and continuing at intervals of five minutes, for one hour and a half. Each portion removed served for acid and bacteriological examinations. He isolated the following organisms:

A. In those nourished with human milk, 85 cases,—*Monila condida*, in 57.6 per cent.; *Bacillus lactis ærogenes*, in 37.6 per cent.; *Oidium lactis*, in 12.9 per cent.; non-liquefying cocci, in 12.9 per cent.; liquefying cocci, in 37.6 per cent.; *Staphylococcus pyogenes aureus*, in 16.4 per cent.; *Bacillus subtilis*, in 11.7 per cent.; and an unnamed delicate bacillus, in 9.4 per cent.

B. In those nourished with cows' milk, 11 cases,—*Bacterium lactis ærogenes*, in 45.4 per cent.; *Oidium lactis*, in 27.2 per cent.; non-liquefying cocci, in 54.4 per cent.; liquefying cocci, in 72.7 per cent.; *Staphylococcus pyogenes aureus*, in 27.2 per cent.; *Bacillus subtilis* in 36.3 per cent.; a delicate bacillus, in 18.1 per cent.; *Bacillus flavescens liq.*, in 27.2 per cent.; and *Bacillus butyricus*, Hueppe, in 100 per cent.

C. No organisms were found in 18 per cent. of the children examined.

In 41 per cent. of the cases examined, the number of organisms found in the stomach did not amount to over 1000 per cubic centimeter, and in only 9 per cent. did the number of organisms amount to more than six million per cubic centimeter.

He came to the following conclusions:

1. The presence of micro-organisms in the stomach is accidental.
2. The number of micro-organisms in the stomach depends directly upon the number in the mouth cavity.
3. Owing to the greater ease of assuming an acid reaction, the suckling's stomach possesses greater germicidal power than does the stomach of the adult.

Abelous (19), in 1889, published the results of his investigations on the bacteria in the stomach. He inoculated plates with stomach contents, by which means he isolated sixteen species, as follows: 1. *Sarcinæ ventriculi*, (2) *Bacillus pyocyaneus*, (3) *Bacterium lactis ætogenes*, (4) *Bacillus mycoides*, (5) *Bacillus subtilis*, (6) *Bacillus amylobacter*, (7) *Vibrio rugula*. The remaining nine species consisted of one coccus and eight bacilli.

The results of his further inquiries, with regard to the action of the gastric juice on the bacteria, and the relation of these organisms to digestion, will be considered under II and III respectively.

Florentini (20), in 1890, while examining the bovine stomach contents for infusoria, mentions that in the third and fourth stomachs, and especially in the latter, may be found large numbers of motile and immotile bacteria, micrococci, and very often also the fungus *Oidium albicans*.

Capitan and Morau (21), in 1890, isolated three distinct species from the human stomach.

Ewald (22), in his book, entitled, "Diseases of the Stomach," published in 1892, mentions that, under certain conditions of abnormal chemical changes in the stomach contents, microscopical examinations of the same will reveal numerous cocci, vibriones, and masses of zoöglea. He does not, however, specify the species that may thus be found.

Gillespie (23), in 1893, published the results of his profound investigations on the "Bacteria of the Stomach." The objects of his investigations were: to study the biochemic properties of the bacteria he isolated from the stomach contents of hospital patients; to estimate the effects of different strengths of hydrochloric acid acting on them; and to deduce from the facts observed any points bearing on the etiology of dyspepsia, and the treatment of that group of diseases. The following is a list of most of the organisms he isolated:

*Bacillus coli communis*, *Sarcinæ ventriculi*, *Mucor*, *Saccharomyces cervisiæ*, *Leptothrix*, *Proteus vulgaris*, *Bacillus subtilis*, *Micrococcus candicans*, *Bacillus luteus* liq., a micrococcus giving an alkaline reaction to bouillon, *Bacillus fluorescens* liq., *Rosa hefe* (pink torula), bacillus (Weisser?), a bacillus liquefying gelatin, a non-motile colon, *Aspergillus niger*, *Bacillus septicus agrigenus*, *Bacterium lactis ætogenes*.

The results he obtained with reference to the behavior of some of these organisms in the presence of different percentages of hydrochloric acid, and with respect to their proteolytic properties, will be considered under II and III respectively.

Since the investigations of Gillespie, no important efforts are reported to have been made to investigate this subject in any of its phases. A few minor observations, however, are on record.



Oppler-Boas (24), in 1895, found an organism associated in gastric carcinoma, which is now known as the Oppler-Boas bacillus.

Straus (25), in 1896, reported that he had cultivated *Bacillus coli communis* from stomach contents, which, in cultures, produced hydrogen sulphide. This organism, however, had already been isolated previously from this situation by Gillespie. The observations of Straus, therefore, are only of a confirmatory nature.

Rosenheim (26), in 1896, reported to have found the fungus *Oidium albicans* in stomach contents. This observation is also only of a confirmatory nature, as the organism had been observed under these conditions at a much earlier date, by Zалensky, in 1864.

Hemmeter (loc. cit.), in 1897, wrote that spores of anthrax may lodge in the mucosa and submucosa of the stomach, giving rise to inflammation, ulceration and necrosis.

From a perusal of the foregoing, it will be seen that though observations on the presence of bacteria in the stomach date back as early as 1842, that since then only three systematic investigations of this subject have been made:

The first of these by van Puteren, 1888, who isolated nine species, consisting of bacteria and fungi; the second by Abelous, in 1889, who isolated sixteen species; and the third by Gillespie, in 1893, who reported to have isolated from eighteen to twenty-four species.

It will also be noted that in all the investigations, up to the present time, the bacteria studied were obtained from the stomach contents of healthy or diseased individuals. From a study of these investigations, it must be inferred that the presence of any species of bacteria in the stomach contents of an individual is accidental, and that no single form may be expected to be constantly present under these conditions.

*(To be Continued.)*

## Note on the Preparation of Culture Media.

Any one who has to prepare nutrient gelatin or agar has experienced difficulty in preventing these solutions from burning when boiled over a free flame. This difficulty is especially troublesome with gelatin, which frequently burns, no matter how much care is taken to prevent it. For the last three years the writer has been in the habit of boiling all culture media in an agate ware double boiler, the outside chamber of which contains a fifty per cent. filtered solution of commercial calcium chlorid. This solution has a boiling point of 112 C. and consequently, when boiling, is hot enough to keep the contents of the inner chamber in rapid ebullition. The advantages of this plan are several. Nothing ever burns. The boiling always takes place quietly, no bumping ever occurring, no matter how much solid matter the solution contains. The rate of ebullition is as easily controlled by adjusting the size of the flame as when direct heating is employed. The time required to prepare gelatin and agar by the method recommended by the Bacteriological Committee of the American Public Health Association may

be shortened, as the preliminary heating in a water bath after the addition of egg albumen becomes unnecessary.

The solution of calcium chlorid is cheap, permanent, and non-corrosive. The writer has used the same solution for nearly two years, merely adding water from time to time to replace that lost by evaporation. It is not even necessary to remove it from the agate boiler.

R. B. F. RANDOLPH, A. C.

Associate Director, Hoagland Laboratory.

## Record Cards for Embedded Material.

The card reproduced herewith is one of the regular catalogue cards, three by five inches, specially printed. Throughout the process of fixing, washing, dehydrating, etc., as well as of collection, the card accompanies the bottle bearing the same number, and at each step the proper item of record—as time—is entered.

No. 96

Material *C. tonsum*, Young leaf.

Fix. Chr—ac 10/10, 9 a. m. 80

Wash. 10/11, 8 a. m. 95

10 100

20 10/12 8:30 Al. & Chl.

30 Chl.

40 Chl. & Par.

50 Par.

60

70



When large quantities of material are being prepared, a temporary memorandum at least has always to be provided for each lot. This card serves such a purpose better than note book or label. And the record once made is in this form easily filed for reference when the material comes up for study. If two or more persons are coöperating, the card, placed perhaps under the bottle on the table, shows what has been done and what is next to be done.

I find that the method both hastens the work and tends to prevent mistakes, whether of record or of procedure.

ROBERT G. LEAVITT.

Ames Laboratory.

## METHODS IN PLANT HISTOLOGY.

CHARLES J. CHAMBERLAIN.

## X.

## BRYOPHYTA.

The Bryophytes, comprising the two groups, Liverworts (Hepaticæ) and Mosses (Musci), present a great diversity of structure, some being so delicate that good preparations are very uncertain, while others are so hard that it is difficult to get satisfactory sections. Between these extremes, however, there are many forms which readily yield beautiful and instructive preparations.

If but one fixing agent should be suggested for the entire group, it would be chromo-acetic acid with three-quarters g. chromic acid and one-half cc. acetic acid to 100 cc. of water. It should be allowed to act for at least twenty-four hours, and perhaps two or three days is better. Always make an effort to get the material into paraffin, using celloidin only as a last resort for refractory structures which resist infiltration and for very delicate structures which persist in collapsing. As one gains in experience and carefulness, the number of cases which seem to demand celloidin will become fewer and fewer.

Instead of treating forms in a taxonomic sequence, we shall consider first the gametophyte structures under the headings *thallus*, *antheridia*, and *archegonia*, and shall then turn our attention to the *sporophyte*.

## HEPATICAE.

Some of the liverworts are floating aquatics, but most of them grow on logs or rocks or upon damp ground. They are found at their best in damp, shady places. Many of them may be kept indefinitely in the greenhouse. *Riccia*, *Ricciocarpus*, *Marchantia*, *Conocephalus*, *Asterella*, and many others vegetate luxuriously and often fruit if kept on moist soil in a shady part of the greenhouse, and they do fairly well in the ordinary laboratory if covered with glass and protected from too intense light. The living plants are very desirable, since they not only furnish the best possible material for habit work and the coarser microscopic study, but they also enable one to secure complete series in the development of the various organs.

*The thallus.*—In many cases it will not be necessary to make a special preparation for the study of the thallus, since preparations of antheridia, archegonia, or sporophytes may include good sections of vegetative portions. This is particularly true of forms like *Riccia*, where the various organs are not raised above the thallus. In forms like *Marchantia*, where the antheridia, archegonia, and sporophytes are borne upon stalked receptacles, it is better to make separate preparations to show the structure of the mature thallus. Sections intended to show the structure of the mature thallus should be  $15\ \mu$  to  $25\ \mu$  in thickness, but sections to show the growing point and development of the thallus should not be thicker than  $10\ \mu$ . Material showing apical cells and development of the thallus

is easily gotten into paraffin even in forms like *Ricciocarpus*, which in their mature condition are in danger of collapsing. The apical region of the foliose Jungermanniaceæ afford an excellent opportunity for studying the development

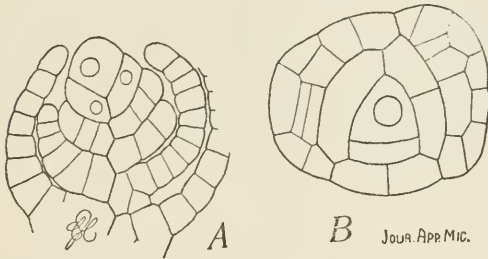


Fig. 1. *Ptilidium ciliare*.  $\times 420$ .

A, longitudinal; B, transverse section of the apex of the leafy gametophyte. Fixed in Flemming's weaker solution, stained in a mixture of acid fuchsin and iodine green. Ten microns.

walls out very sharply. Chromo-acetic acid followed by Delafield's hæmatoxylin or Bismark brown is good for apical cells and developing regions, but a light counter stain with erythrosin improves preparations of the mature thallus. After corrosive sublimate-acetic the material may be stained in bulk with alum cochineal or alum carmine, thus giving fairly good preparations and saving considerable labor.

*Antheridia*.—If you have the material it is not difficult to get good preparations showing the development of antheridia. In forms like *Asterella*, *Pellia*, etc., cut out a small portion of the thallus bearing the antheridia. The piece should not be more than a quarter of an inch square, and if it can be smaller, so much the better. For early stages of the antheridia of *Marchantia*, select young antheridiophores which still lie close to the thallus. These readily cut as thin as  $5\mu$ , and a single slide will usually show a more complete series than is represented in the figure of *Asterella*,

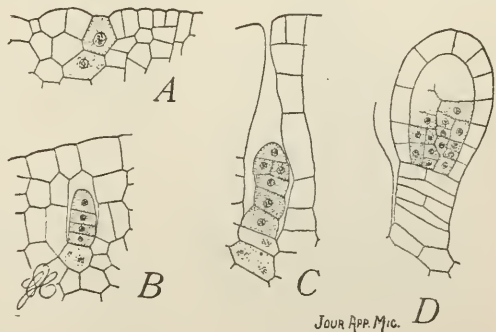


Fig. 2. *Asterella hemisphærica*.  $\times 255$ .

Successive stages in the development of antheridia. Fixed in chromo-acetic, stained with Delafield's hæmatoxylin. Section ten microns thick.

than that represented in D, showing the development of the spermatozoid, the paraffin must be rather hard (melting at  $55^{\circ}\text{C}$  to  $65^{\circ}\text{C}$ ), and the sections should not be thicker than  $5\mu$ , while  $2\mu$  or  $3\mu$  is best. For such stages use the safranin-gentian violet-orange combination, Heidenhain's iron alum hæmatoxylin



with or without a faint trace of erythrosin or orange G, or use a mixture of acid fuchsin and methyl green. Nothing but practice and patience will bring success in such critical work.

If antherozoids are found escaping, transfer them to a small drop of water on a clean slide, invert the drop over a one per cent. solution of osmic acid for two or three minutes, allow the drop to dry up, pass the slide through the flame two or three times as in mounting bacteria, and then stain sharply in acid fuchsin. This should show the general form of the antherozoid, and will usually bring out the cilia.

*The archegonia.*—The methods for archegonia are practically the same as for antheridia. Too much stress cannot be laid upon the importance of carefully selecting the material.

Use very small pieces, and before placing them in the fixing agent trim them to such a shape that the position of the archegonia will be accurately known even after the pieces are imbedded in paraffin. For stages like A and B, Delafield's hæmatoxylin is a good stain, and  $10\ \mu$  is about the right thickness. For stages like C, in such forms as *Marchantia*, where the necks are long and often somewhat curved, it is better for general purposes to use sections from  $15\ \mu$  to  $20\ \mu$  in thickness. If it is desired to obtain preparations showing the cutting off of the ventral canal cell, the development of the oösphere and the process of fertilization, the sections should be from  $5\ \mu$  to  $10\ \mu$  in thickness and the same staining may be used as for the development of antherozoids. For archegonia containing young embryos, like that shown in D, Delafield's hæmatoxylin without any counter stain is to be recommended.

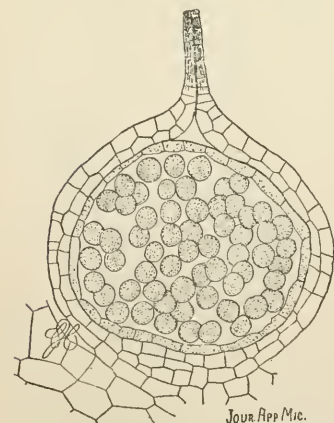


Fig. 4. *Ricciocarpus natans*.  $\times 104$ .  
Young sporophyte enclosed in the archegonium. Spore-mother-cell stage. All the cells of the sporophyte except a single peripheral layer (dotted in the figure) produce spores. Fixed in picro-acetic acid and stained in Delafield's hæmatoxylin. Celloidin section 30 microns in thickness.

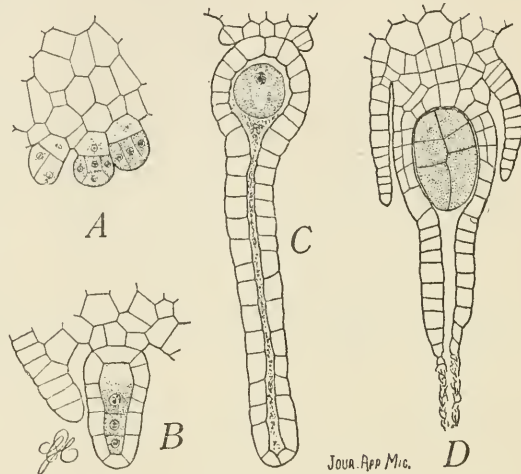
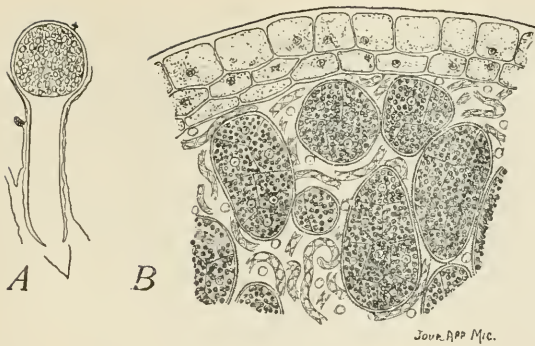


Fig. 3. *Marchantia polymorpha*.  $\times 400$ .

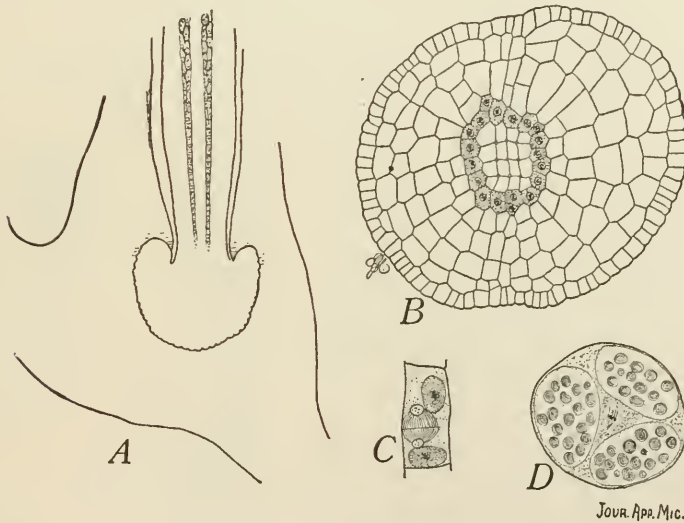
- A. Three early stages in the development of the archegonia. Delafield's hæmatoxylin. B. Young archegonium showing two neck canal cells and the central cell before the cutting off of the ventral canal cell. Fuchsin and methyl green. C. Mature archegonium just ready for fertilization. Safranin-gentian violet-orange. D. Young embryo. Delafield's hæmatoxylin.

*The sporophyte.*—Sporophytes in early stages of development often yield good preparations without very much trouble, but in later stages they are frequently difficult to cut on account of the secondary thickening of the capsule wall,

Fig. 5. *Peltia epiphylla*.

A. Habit sketch of sporophyte.  $\times 10$ . B. Small portion of sporophyte (at  $\times$  of A), showing the capsule wall, the spores, and the elaters. Fixed in chromo-acetic acid and stained in cyanin and erythrosin. Ten microns.

and the stubborn extine of the mature spores. It is hard to get *Ricciocarpus* into paraffin without shrinking, and the same thing may be said of other forms which have such loose tissue with large air cavities. For stages like that shown in the figure, as well as for older sporophytes, it will be found more satisfactory to use celloidin, and cut the sections from  $20\ \mu$  to  $30\ \mu$  thick. However, if nuclear details of the development of spores are to be studied, celloidin sections cannot be cut thin enough, and, besides, they do not allow the stains required in cytological work. Forms like *Peltia* cut well in paraffin, especially in younger stages, but even in

Fig. 6. *Anthoceros laevis*.

A. Longitudinal section of lower portion of sporophyte imbedded in the gametophyte.  $\times 45$ . B. Transverse section of lower portion of sporophyte.  $\times 200$ . Delafield's hæmatoxylin. Ten microns. C. Vegetative cell from lower portion of the sporophyte.  $\times 560$ . Fixed in Flemming's weaker solution and stained in a mixture of acid fuchsin and iodine green. Five microns. D. Spore-mother-cell showing three of the four chloroplasts with numerous starch grains. The nucleus is in the metaphase of the first division.  $\times 560$ . Fixed in Flemming's weaker solution, stained in safranin-gentian violet-orange. Five microns.

case of mature sporophytes it is not necessary to resort to celloidin. In *Peltia* and *Conocephalus* the spores are very large and have a rather thin wall. Both these genera show a peculiar, intrasporal development of the gametophyte, i. e., the gametophyte develops to a considerable extent before it ruptures the spore

wall. For sporophytes of *Marchantia* it is better not to cut the whole receptacle, but rather to remove the branches so that they may be cut separately. For the very best preparations of mature sporophytes it will pay to trim away the gametophyte structures, leaving only enough to show the foot with a few of the surrounding cells. Sections  $5\mu$  or  $10\mu$  thick can be made without much difficulty from material prepared in this way.

Among the Bryophytes no other form affords such an excellent opportunity for studying the development of spores as *Anthoceros* since a single longitudinal section of the sporophyte may show all stages from earliest archesporium to mature spores. For studies like A and B, chromo-acetic material cut  $10\mu$  thick and stained in Delafield's hæmatoxylin is very good, but to bring out details of the chloroplast Flemming's weaker solution gives better results. The starch grains in the chloroplasts take a beautiful violet color with the safranin-gentian violet-orange combination. It is very difficult, however, to bring out the details of nucleus or chloroplast on account of the minute size of these structures. The drawings from which C and D were reproduced were made with a one-sixteenth oil-immersion objective. The drawings, like all the others illustrating the Bryophytes, were reduced one-half by photography.

#### MUSCI.

Material for a study of the mosses is much more abundant, and a series of stages in the development of the various organs is easily secured, but it is much more difficult to obtain good preparations because so many of the structures are hard to cut. Chromo-acetic acid is to be recommended as the most satisfactory fixing agent, but where structures are refractory and very likely to make trouble in cutting, it will often be found more satisfactory to use picric-acetic acid in the 70 per cent. alcohol, since material fixed in this reagent does not become as hard or as brittle as that fixed in any of the chromic acid series.

*Antheridia*.—It is easy to find material for a study of antheridia, because, in so many cases, the antheridial plants can be detected at once without even a pocket lens. *Funaria*, with its bunch of antheridia as large as a pin head, is extremely common everywhere. Spring is the best time to collect it, but it is found fruiting in the autumn and sometimes in summer; besides, it is easily kept in the greenhouse, where it may fruit at any time. *Mnium* has a still larger cluster of antheridia, which may be seen at a distance of several yards. *Polytrichum* also has a large cluster of antheridia surrounded by reddish leaves, so that the whole is sometimes called the moss "flower." In making preparations of *Polytrichum* these colored leaves should be carefully

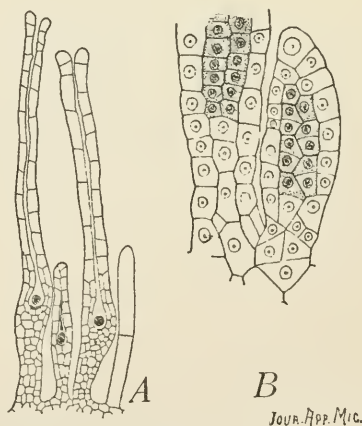


Fig. 7.

A. Archegonia of *Webbera candidans*.  $\times 104$ .  
Celloidin section, 20 microns. B. Young  
antheridia of *Polytrichum commune*.  $\times 420$ .

removed after the material has been gotten into 70 per cent. alcohol. A single antheridial plant of *Polytrichum* often furnishes a fairly complete series of stages in the development of antheridia. In all cases the stem should be cut off close up to the antheridia, for many of the moss stems cut like wire. It is not necessary to use celloidin for antheridia, nor is it desirable, except where sections from  $20\ \mu$  to  $50\ \mu$  thick are wanted for habit work.

*Archegonia*.—Since the necks of the archegonia are usually long and more or less curved, it is necessary, for habit work, to cut sections as thick as  $20\ \mu$  or  $30\ \mu$  in order to get a view of an archegonium in a single section. Celloidin is better for such preparations, but for the development of the archegonium, the oosphere, the canal cells, and also for the process of fertilization, it is better to

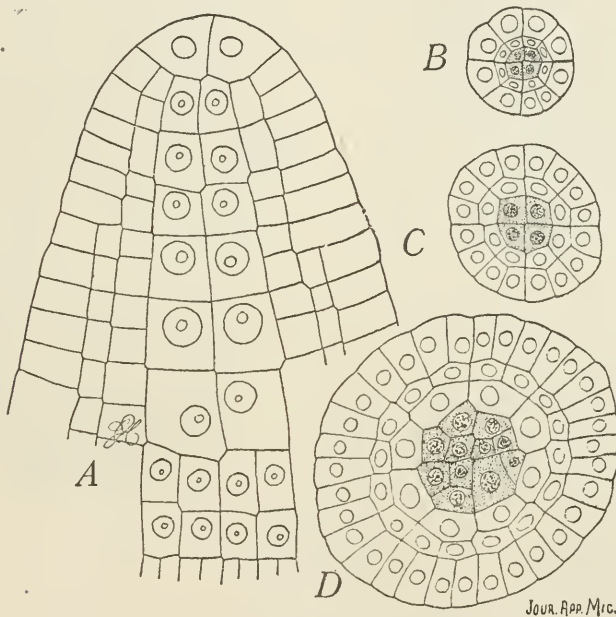


Fig. 8. *Funaria hygrometrica*.

A. Apex of young sporophyte showing endothecium and amphithecium.  $\times 420$ . Chromo-acetic acid and Delafield's hæmatoxylin. Ten microns. B, C, and D, transverse sections of a sporophyte of the same age as A, taken at three different levels.  $\times 255$ . Ten microns.

use paraffin. For the thick celloidin sections the material may be stained in bulk in alum cochineal, but thin paraffin sections should be stained on the slide with more critical stains.

*The sporophyte*.—It is often difficult to get good mounts of sporophytes. In the younger stages the calyptras are likely to interfere with cutting, while in the older stages the peristome or hard wall of the capsule occasions the trouble. If an attempt is made to remove the calyptra in young stages, like A of the figure, the apex of the sporophyte usually comes with it. While picro-acetic acid material cuts more easily, chromo-acetic acid followed by Delafield's hæmatoxylin gives so much sharper differentiation in stages like those shown in Fig. 8,



that it is better to use harder paraffin ( $55^{\circ}$  to  $60^{\circ}\text{C.}$ ) and make an effort to get preparations from chromic material.

Stages like that shown in Fig. 9 are cut with comparative ease, for the calyptra is easily removed and the capsule wall is not yet hard enough to occasion any difficulty. The cell walls are so easily stained in moss capsules that a light counter stain with erythrosin or acid fuchsin may be used to bring out the cytoplasm and plastids without appreciably obscuring the cell walls. *Funaria* and *Bryum* afford an excellent study in the development of the capsule, since all the structures of a highly differentiated moss sporophyte are present, and *Bryum* is particularly easy to cut in stages like those shown in Fig. 10.

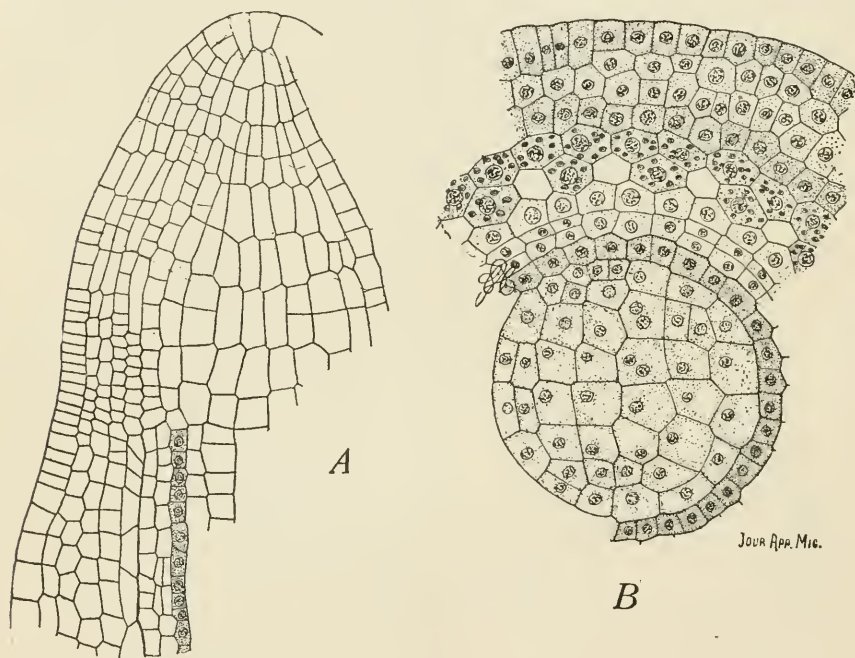


Fig. 9. *Funaria hygrometrica*.  $\times 420$ .

A. Longitudinal section of capsule. B. Transverse section of capsule of about the same age as A. The columella, archesporium, outer spore case, two layers of chlorophyll-bearing cells, and the beginning of the air spaces can be distinguished at this stage. Delafield's hæmatoxylin and erythrosin. Ten microns.

Sporophytes, in their more mature stages, are almost sure to present considerable difficulty in cutting. For general work fairly good preparations may be gotten from celloidin material, but it is worth while to try paraffin, for it is sometimes successful, and when it does succeed it is far superior. As soon as the cell walls begin to thicken, as in the development of the peristome, safranin is an excellent stain and this, followed by Delafield's hæmatoxylin, will give an elegant differentiation in the older stages of the sporophyte.

The mature sporophytes of *Sphagnum* are exceptionally hard to cut. It will be worth while to prick the capsule with a needle when the material is collected. This will allow the fixing agent to penetrate readily, and will also facilitate the

infiltration of paraffin or celloidin. The puncture causes only a slight damage, and need not reach the really valuable portion which is to furnish the median longitudinal sections.

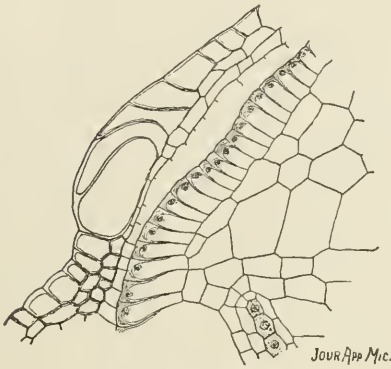


Fig. 10. Bryum.  $\times 200$ .

Portion of a nearly mature capsule showing operculum, annulus, peristome, and three cells of the sporogenous tissue. Fixed in Flemming's weaker solution, stained in safranin and Delafield's hæmatoxylin. Fifteen microns.

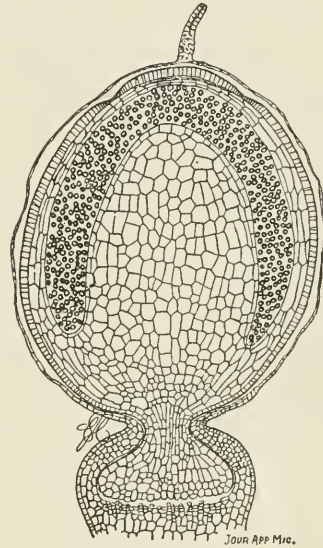


Fig. 11. Sphagnum.  $\times 24$ .

Longitudinal section of mature sporophyte showing also the upper portion of the pseudopodium and the calyptra. Chromo-acetic acid, Delafield's hæmatoxylin. Paraffin. Ten microns.

Protonema and teased mounts of antheridia and archegonia may be made directly in 50 per cent. glycerine without fixing or staining. They will keep the green color for a long time.

(To be continued.)

**GLYCERINE JELLY.**—This medium, available for such a variety of purposes, may be easily made in the laboratory. To insure the greatest possible transparency, the gelatin should be thoroughly washed and it should not be subjected to high temperatures. Use any good photographic gelatin, place it in apparatus commonly used for washing laboratory specimens, and leave it in gently-running water over night. By the time it is perfectly clean, the gelatin will have absorbed as much water as possible, and it may then be melted by gentle heat in an equal quantity of pure glycerine. After this it should be filtered at least three times. This is done by heating the funnel in hot water, after which the filter paper is placed in position and hot water poured through it before filtering the jelly. A crystal of thymol will keep it from molding.

Glycerine jelly made in this way is clear and may be used for mounting *toto* specimens in museum jars, as well as for mounting microscopical objects, etc.

# Journal of Applied Microscopy.

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L. B. ELLIOTT, EDITOR.

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processes and apparatus employed in converting an object—macroscopic or microscopic—into an illustration. As will be seen at a glance, this field is very wide, including as it does everything from photography of a plant, animal, or rock in the field, to the projection of the finished picture on the screens or its preservation on the printed page. The matter will be selected with great care and only practical methods will be admitted. Fine enamel paper will be used, permitting the printing of abundant illustrations. The JOURNAL for 1900 will be larger than heretofore and will contain many useful papers and series of articles, among others, the conclusion of Prof. Chamberlain's papers on methods in botanical microtechnique, Mr. Weiss's exhaustive studies on the bacteriology of the cat's stomach, and Prof. Macbride's paper on methods of studying slime moulds. Prof. Chamot of Cornell University will contribute a series on the equipment of the micro-chemical laboratory and the micro-chemical technique. A series of papers descriptive of the laboratories of the University of Chicago, University of California, Leland Stanford Jr. University, Adelbert College, Hendryx Laboratory at Los Angeles, California State Normal School, and others, will also be one of the features of the year. And many other interesting and valuable papers are already in our hands for publication.

\* \* \*

DURING the past two years we have used extraordinary efforts to introduce the JOURNAL to the notice of every person in America interested in work requiring the use of the microscope, and of adding every such person to our subscription list, knowing that unless a large number of subscribers could be secured the enlargement and improvement of the JOURNAL according to our plans could not be accomplished. Our publishers have been extremely liberal in supplying the necessary copies for this work, which has been done thoroughly. This liberality has, in some instances, been mistaken for an intention to distribute the JOURNAL gratuitously. Such is not the case. There is no free list, and the JOURNAL is dependent for its existence on whether it can be self-supporting or not. It is therefore to be hoped that persons interested will subscribe promptly.

\* \* \*

THE index for 1899 will be sent out with the January, 1900, number, and will consist of a general index, an author's index, and a list of papers noticed in the review department. Our subscribers would do well to bind their volumes, as only a few sets of our stock of back numbers remain, and the volume for 1899 will soon be out of print.

THE present number concludes the second volume and second year of the JOURNAL OF APPLIED MICROSCOPY. We take this occasion to extend our thanks and those of our readers to the contributors who have during the past year furnished so many valuable communications. Evidence is not wanting that this work is not only appreciated, but put to practical use.

\* \* \*

WITH the beginning of Volume III another important department will be added, a Department of Laboratory Photography, in which it will be our aim to supply information regarding the

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN.

Books for review and separates of papers on botanical subjects should be sent to  
Charles J. Chamberlain, University of Chicago,  
Chicago, Ill.

## REVIEWS.

**Underwood, Lucien M.** Moulds, Mildews, and Mushrooms; a guide to the systematic study of the Fungi and Mycetozoa and their literature. 8vo. pp. V+236, 10 plates. Henry Holt & Co., New York, 1899. \$1.50.

Probably there are but few teachers of botany in our larger institutions who have not been called upon to recommend some book which would enable

the inquirer to identify the common fungi, but the answer has necessarily been that there was no book in English covering this ground. Consequently, the present work cannot fail to be welcomed by teachers and students alike. Of course, all the fungi could not be treated within a reasonable space, and so the author has selected the more conspicuous fleshy and woody fungi, the cup fungi, and genera containing parasitic species. The headings of the chapters will give an idea of the scope of the work. They are: (1) Introductory. (2) Relations of Fungi to Other Plants. (3) Reproduction, Constituents, and Habits. (4) Phycomycetes. (5) Ascomycetes. (6) Fungi Imperfecti. (7) The Lower Basidiomycetes. (8) The Higher Basidiomycetes. (9) Myxomycetes. (10) The Study of Mycology in General, and its Study in America in Particular. (11) Geographic Distribution of American Fungi. (12) Methods of Collection and Preservation of Fungi. Hints for further study. There are indices to Latin names, host plants, authors and collectors, and also a general index and explanation of terms.

The keys are clear and will enable the average student to determine the genera. There are also descriptions of species for the edible and the poisonous forms. In arrangement the writer has followed *Die natürlichen Pflanzenfamilien* but with some change in the sequence of groups. The names of orders and families conform to the system proposed at Berlin.

The book can be most heartily recommended not only to the epicure who desires to eat with confidence, but also to the busy teacher and student who feel the need of a ready means for identifying the common fungi.

C. J. C.

**Rusby, Henry H., and Jelliffe, S. E.** Morphology and Histology of Plants; designed especially as a guide to plant analysis and classification, and as an introduction to Pharmacognosy and Vegetable Physiology. 8vo., pp. XI+378, 693 illustrations. Published by the authors., New York., 1899.

The fact that the large edition of their *Essentials of Pharmacognosy*, which the present book replaces, was so soon exhausted, has shown a demand for a work of this kind, and has encouraged the authors to publish a more com-

prehensive text. The present volume includes all the subject matter of the *Essentials*, and also a large amount of more general information. The topics of the previous book have been treated with more completeness, and there have been added chapters on floral dissection and analysis, descriptions of



flowers by means of diagrams, morphology, and classification of cryptogams, on nomenclature, and on the collection and preservation of specimens.

The first part of the book, dealing with morphology, is occupied largely by descriptions of the flower, seed, root, stem, and leaf.

The second part, devoted to histology, gives descriptions of the cell, cell contents, reactions of cell constituents, and an account of the tissues of higher plants, with particular reference to features of special interest to students of pharmacognosy.

In both parts the illustrations are numerous and very many of them are original.

C. J. C.

**Karsten, George.** Die Diatomeen von Kiehl-er Bucht. Wissenschaftliche Meeresuntersuchungen. Herausgegeben von der Commission zur Untersuchung der deutschen Meere in Kiel und der Biologischen Anstalt auf Helgoland. Abth. Kiel. N. F., IV, 19-295, 219 text figures, 1899.

This is one of the most important contributions to the literature of Diatoms which has yet been made. The work must be regarded not merely as a guide for the determination of the species of a limited locality, but rather

as a comprehensive text-book of diatom lore. Twenty-eight genera and over 200 species are described. Here the author has departed from the usual, comparatively superficial methods, and has taken into account the form and structure of the protoplast, the position of the nucleus, the number, form, and position of chromatophores, the occurrence of pyrenoids, and finally the complete life history of each species as far as this has been possible.

The second part of the work, dealing with the structure and development of Diatoms, consists of six chapters:

I. The Diatom Cell. A study of cell characters convinced the author that the number and position of the chromatophores is the most important taxonomic character, and that mere frustule structures are not sufficient for determining the limits of species. II. Cell division. III. Movements of diatoms. The raphe is regarded as the organ of locomotion. IV. An attempt to explain the variety of form of diatoms, by their relation to environmental factors. V. The auxospores. VI. The rôle of the Diatomaceæ in the economy of nature. There is also an extensive bibliography.

C. J. C.

#### RECENT LITERATURE.

**Biffen, R. H.** On the Biology of *Agaricus velutipes*. Jour. Linn. Soc. Bot., **34**: 147-162, pls. 2-4, 1899.

**Ward, H. Marshall.** *Onygena equina*, Willd., a Horn-Destroying Fungus. Phil. Trans. of the Roy. Soc. of London, **191**: 269-291, pls. 21-24, 1899.

**West, W.** Some Oscillarioideæ from the Plankton. Jour. of Bot. British and Foreign, **37**: 337-338, pl. 400, 1899.

**West, W., and West, G. S.** Fresh Water Algæ of West Indies. Jour. Linn. Soc. Bot., No. 258, 1899.

**West, G. O.** Variation in the Desmidiæ. Jour. Linn. Soc. Bot., No. 257, pls. 4, 1898.

**Wuiczki, C.** Ueber die Befruchtung bei den

Coniferen. 8vo. 57 pp., pl. 1., Warschau, 1899. (In Russian.)

**Slavicek, Fr. J.** Zur Kenntniss der Keimlinge zumeist fremdländischer Coniferen. Verhandlungen der Forstwirthe von Mähren u. Schlesien. Heft. 2, 47 pp., 1899.

**Taylor, L. J.** The Scope of Natural Selection. Nat. Science, **15**: 114-129, 1899.

**Kuckuck, P.** Ueber Polymorphie bei einigen Phæosporeen. Festschrift für Schwendener, p. 357, pl. 13, 1899.

**Macbride, Thomas H.** The North American Slime Moulds; being a list of all species of Myxomycetes hitherto described from North America, including Central America. 8vo., pp. XVII+269, 18 pls. Macmillan, 1899.

## ANIMAL BIOLOGY.

AGNES M. CLAYPOLE.

Separates of papers and books on animal biology should be sent for review to  
 Agnes M. Claypole, Sage College,  
 Ithaca, N. Y.

## CURRENT LITERATURE.

**Dimmer, F.** Eine Modification der Cellöidin-serienmethode. Zeitschr. für wiss. mikros., 16: 44-46, 1899.

The author used the method described for celloiden material stained in toto or for preparations to be stained by the

Weigert or Weigert-Pal process. For the latter cases the author attempted to find a process by which the coating of the celloidin sections with two layers of collodion could be avoided, in this way leaving one side free for the penetration of stains. Obregia poured a sugar solution over a glass plate on which the sections could be placed. This plate so prepared was treated with a solution of photoxylin, which dissolved off, the sugar and the sections contained in the coating of photoxylin can be lifted from the glass together. Instead of the sugar, the author used a gelatin solution (gel. 16 g., warm water 300 g.). Large glass plates previously warmed were coated thinly, and laid horizontally, and protected from dust till dry, which was usually in about two days. The sections were transferred in the usual way with tissue paper to the glass prepared in this way, and washed over with seventy per cent. alcohol; this is absorbed with paper and the sections well pressed down to the glass at the same time. A piece of silk paper for naming the sections can be fastened on at the same time in the same way. Photoxylin solution (photox. 6 g. absol. alcoh. and ether <sup>aa</sup> 100 cc.), is poured over all and allowed to dry off somewhat; the plate is then placed in a vessel with water of from 50° to 55° C, after scraping the photoxylin layer from the edge of the glass with a sharp knife to allow the water to penetrate and dissolve out the gelatin. The sections fastened together with photoxylin are readily separated from the glass, and can be transferred to stains by means of tissue paper, and eventually carried through clearer. If the photoxylin is not easily separated from the glass, longer treatment with warm water will loosen it.

The process is easy and simple; the gelatin wash to the glass is quickly done and dried, and the sections if well pressed down are not disturbed by the pouring on of photoxylin. This process, as already mentioned, is only for toto stained preparations or those to be treated by the Weigert method. In other stains the small amount of gelatin remaining colors so as to spoil the preparation.

A. M. C.

**Rosin, H.** Ueber ein neue Gruppe von Anilinfarbstoffen, ihre Bedeutung für die Biochemie der Zelle und ihre Verwendbarkeit für die Gewebsfärbung. Berliner klin. Wochenschr., pp. 251, 1898.

Rosin states that an entirely new staining material is made by mixing solutions of acid eosin and basic methylin-blue, which he calls "acid-eosin-methylin-blue."

In a similar way he obtains a new series of crystallized stains by the union of other acid and basic anilin colors. To these belong the substance

described by Erlich as formed by a mixture of acetic acid rosaniline and ammonium-picrate. To quote from Erlich: "If concentrated aqueous solutions of an acid and alkaline anilin dye be mixed so that the resulting mixture is neutral, or nearly so, a precipitate appears which, when the proportions are right, is abundant, but is soluble in excess of either the acid or alkaline dye." By mixing a solution of eosin or erythrosin and methylin-blue, of methyl-orange and methyl-green, of rubin and malachite-green, of picric acid and methylin-blue or magenta-red, such precipitates are formed which are almost insoluble in water, but can be dissolved in alcohol either directly or from alcoholic solutions obtained by concentration of the solution or by the addition of water of crystallization. The author especially values the mixture of eosin acid and methylin-blue for biochemical purposes. The color of the liquid is changed from blue-violet or green fluorescent to pure blue or blue-green, a return to the old color being possible by the use of organic acid, but not by mineral acids. Excess of alkalinity changes the color to red, but neutralizing restores it. All acid substances are colored blue, all alkaline red, all neutral violet. Hence celloidin, mucin or nuclein stain blue, albumin, fibrin red. In tissue sections the nuclei are blue, the protoplasm red, except with nerve cells in which the ground substance of the protoplasm is rose-red, but Nissel's granules blue and the nuclei not blue. Since the substances thus formed by mixtures are largely insoluble in water, only alcoholic solutions are available, which serves well in leucæmia and malaria. A comprehensive application of these stains is possible, the "eosin-acid" methylin-blue dissolves in a solution of methylin-blue or eosin better than in water, and the more according to a greater concentration of the liquids. These solutions are not then neutral, but basic with methylin-blue and acid with eosin, but they contain the compounds necessary in saturate solutions. These solutions are particularly good in either diluted or aqueous solutions for tissue staining or the demonstration of nuclear chromatin. By the combination further of neutral stains another new series of anilin dyes is obtained, which have a theoretical as well as practical significance.

A. M. C.

## RECENT LITERATURE.

- Lewertsoff, A. N.** Studien zur Entwicklungsgeschichte des Wirbelthierkopfes. I.
- Nocht,** Zur Färbung der malariaparasiten. Centralbl. f. Bacteriol. **25**: 764-769, 1899.
- Feinberg,** Ueber Amöben und ihre Unterscheidung von Körperzellen. Fortschr. d. Med., **17**: 121, 1899.
- Bordas, L.** Recherches sur les organes de la Génération de quelques Holothuries. Ann. Fac. des sc. de Marseille, **9**: 187-207, 1 pl.
- Fauvel, Pierre.** Les stades post larvaires des arenicoles. Proceed. of the Fourth Internat. Congress of Zoöl. Cambridge, pp. 229-230, 1898.
- MacBride, E. W.** The Development of Echinoids. Part 1, The Larvæ of Echinus miliaris and E. esculentus. Quart. Journ. of Micros. Sci. No. 167, 335-339, 1 pl., 1899.
- Martin, H.** Étude de l'appareil glandulaire venimeux chez un embryon de Vipera aspis. Bull. Soc. Zoöl. de France, **24**: 106-116, 13 figs.
- Rabaud, Etienne.** Sur le parablaste et l'endoderme vitellin du blastoderm de Poule, Compt. Rend. Acad. Sc. **129**: 167-168, 1899.
- Schaudinn, F.** Ueber den Generationswechsel der Coccidien und die neuere Malariaforschung. Sitzungber. d. Ger. Naturforsch. Freunde, Berlin. **7**: 159-178, 1899.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. H. WAITE,  
University of Michigan.

Separates of papers and books on bacteriology should be sent for review  
to H. H. Waite, 709 North University avenue,  
Ann Arbor, Michigan.

**Councilman, W. T., Mallory, F. B., and Wright, J. H.** Epidemic Cerebro-Spinal Meningitis and its Relation to other Forms of Meningitis. Report of State Board of Health of Mass., 1898.

This contribution embraces the clinical history of 111 cases. Of thirty-five cases on which a post-mortem examination was made, in all but four cases

the diplococcus intracellularis meningitidis was found, and in the four cases in which it was not found post-mortem, it was found in one in the fluid obtained by spinal puncture previous to death. In two of the three remaining cases in which it was not found, the disease was chronic, and no acute lesions were found. The fourth of the series was chronic and complicated with a mixed tuberculous infection.

Though the diplococci were found frequently in great numbers, both on examinations of streak preparations and in sections, yet frequently it was exceedingly difficult to obtain cultures from these cases. In only two cultures, one from the cord, and the other from the brain, of ten cultures made from the latter, and nine from the cord, was the organism found in a single instance, though the diplococcus was found in abundance in streak preparations and in sections. Cultures are much more easily obtained in acute than in chronic cases.

According to Councilman, Mallory, and Wright, the diplococcus intracellularis meningitidis has the following characteristics: "It is a micrococcus of the same size as the ordinary pathogenic micrococci, and appears in diplococcus form as two hemispheres separated by an unstained interval. It stains with any of the ordinary stains for bacteria, and is decolorized by the Gram method of staining. There is considerable irregularity in staining, some organisms being brightly stained, others more faintly. Sometimes this difference in staining is seen in a single pair of organisms, one being more brightly stained than the other. There may also be considerable variation in size, and the larger organisms stain imperfectly. In the swollen organisms there is often a brightly stained spot in the center, while the remainder of the cell is scarcely colored. It may have been this condition which was mistaken by Jäger for a capsule. These variations in size and staining appear to be due to degeneration, and are more common in old than in fresh cultures."

"The two organisms are usually sharply separated, but in some there seems to be a small amount of material uniting them. Division takes place usually in one plane giving rise to diplococci; tetrads are occasionally seen. There is little or no tendency to growth in the streptococcus form, although short chains of four to six organisms may be found. We have never seen the streptococcus formation described by Jäger, and in the short chains the longitudinal line on which he lays much stress was not seen."



The germ does not grow profusely on any medium. The blood-serum mixture of Löffler, prepared according to Mallory, appears to be best adapted for its growth. It grows but feebly on agar, and in many cases there is complete failure of growth, especially in cultures from the fluids and tissues of the brain and cord. Under no case is the growth abundant, but it is characterized by isolated colonies which are few in number. To keep up pure cultures it is necessary to make transfers almost daily on several tubes of medium, and even then, on some of them, growth would not take place.

Grown on Löffler's blood-serum, the colonies sometimes (especially in acute cases with many organisms present) are minute, round, and transparent, bearing a striking resemblance to Fränkel's diplococcus; in other cases growth is in round, whitish, viscid looking colonies, with smooth, sharply defined outlines which tend to become confluent. Growth on plain agar is feeble, better on glycerine agar, best on blood-serum. The colonies on glycerine agar resemble the colonies on blood-serum, are pearly in color and translucent. On other media growth is poor and not characteristic.

In the tissues the diplococcus is found almost exclusively in the polynuclear leucocytes, but never in other cells. It has no definite position in the leucocyte, and is not found in the nucleus, as has been previously described by other investigators. The number in the cell varies from one pair to so many that the nucleus is obscured.

The organisms seem to be strictly confined to the lesions of the disease and are never found in the blood, liver, spleen, and kidneys, and never cause septicemia. Mixed infections occurred, the pneumococcus being found seven times associated with Friedländer's bacillus in one instance. Occasionally streptococci and staphylococci were found.

In the lower animals subcutaneous inoculation is negative. In some cases intraperitoneal and intrapleural inoculation of pure cultures, as well as of the fluid obtained by lumbar puncture in guinea pigs and rabbits, showed many germs present in the pus cells, from the exudate in the pleural and peritoneal cavities, but there was no invasion of the tissues. The only successful inoculation resulting in the production of typical meningitis was made on a goat, the animal being inoculated in the spinal canal with one cc. of a bouillon suspension of a pure culture of the diplococcus from an acute case at the Massachusetts General Hospital.

The authors of this contribution conclude that epidemic cerebro-spinal meningitis is an acute infectious disease, produced by a micrococcus characterized by its growth in pairs, and by certain cultural and staining properties. The organism is one of feeble vitality and grown with difficulty. The essential abode of the organism in disease is in the meninges of the brain and cord. The organism may gain access to the brain by the lymphatics of the mucous membrane of the nose, but this has not been demonstrated. The organism does not produce a general septicemia, nor is there a general invasion of the tissues. The organism may enter the lungs and produce a focal pneumonia. Most sporadic cases are due to organisms other than the meningococcus, though sporadic cases probably do occur due to the meningococcus in places where an

epidemic has previously occurred. Of the organisms found in sporadic cases the tubercle bacillus, the pneumococcus, and streptococci seem to be the most frequent causes. Streptococci or staphylococci may be associated with one another or with other pus organisms. In the majority of these cases the infection of the meninges is apparently secondary to lesions in some other part of the body. The best method of diagnosis is by lumbar puncture, a microscopical examination being made of the fluid and cultures on blood-serum.

H. H. W.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

RICHARD M. PEARCE, M. D.

Harvard Medical School, Boston, Mass., to whom all books and papers on these subjects should be sent for review.

**Hüffel.** Die Vaccinekörperchen. Nach Untersuchungen an der geimpften Hornhaut des Kaninchens. Zweites Supplement heft der Beiträge zur Path. Anat. und Allg. Path. 4 plates., 1899.

This work deals with the bodies found in the epithelial cells of the rabbit's cornea after inoculation with vaccine virus.

Seventy-six rabbits were inoculated, and from these 24,000 sections were prepared. The virus was obtained from four-day-old pustules on calves. The corneæ were studied fresh, and after fixation in Flemming's fluid and in corrosive sublimate. Biondi's triple stain was used for most preparations.

Control experiments consisting in mechanical irritation and injection of pyogenic cocci were negative in all of the twenty-two rabbits tried.

Within twenty-four hours after inoculation the vaccine bodies could be found accompanied, after six hours, by a slight invasion of leucocytes.

During the next eighteen hours the number of vaccine bodies and of leucocytes increased rapidly, and the epithelium became much swollen. The vaccine bodies appeared first around the inoculation wound and then spread peripherally.

These bodies are drop-like, spherical, 1.5–5 $\mu$ . in diameter, have no envelop or border, and are not surrounded by any granules or thread-like membrane. They stain deeply with nuclear dyes, do not decolorize easily. With Biondi's stain they are colored a dark blue. They appear first in depressions in the nucleus and later in the protoplasm, and vary greatly in number and size.

A second form is surrounded by a homogeneous mantle of variable thickness which stains red.

A third, and the most abundant form, is surrounded by red granules and varies much in size.

In a fourth form, delicate, somewhat granular filaments extend out from the central body, and are inserted into the surrounding protoplasm of the cell.

Half moon, sickle, and spindle shape forms were also occasionally seen.

Fifteen corneæ examined on warm stage showed bodies similar to those seen in stained section. No change of form or evidence of mobility was seen. These bodies disappear when treated with a saturated solution of sodium chloride, but come out sharply when in contact with a five per cent. solution of acetic acid.

The writer believes these bodies to be derived from the protoplasm of the epithelial cells as the result of the specific action of the vaccine virus. This explanation is opposed to that of Salmon, who believes them to be masses of chromatin derived from leucocytes, and to that of Guarnieri and L. Pfeiffer, both of whom regard them as protozoa which are multiplying in the tissues. Still others think they are formed from the nuclei of epithelial cells.

The writer says that while his work throws no light on the nature of vaccine virus, it clears up the nature of the so-called vaccine bodies.

The work is beautifully illustrated by four double-page colored plates containing 179 figures.

R. M. P.

**Birch-Hirschfeld, Arth., und Garten, S.** Ueber das Verhalten in plantirter embryonaler Zellen im erwachsenen Thierkörper. Beiträge zur Path. Anat. und Allg. Path., 26-1, p. 132, 2 plates.

Cells of young embryos were introduced into adult animals of the same species. Rabbits and their embryos were used chiefly, but chickens, goats,

salamanders, and frogs were also tried.

Very young embryos were used on account of the greater capacity of their cells for proliferation, and also because it was desirable to determine whether it was possible for a differentiation of cells to take place after transplantation.

The embryos were teased out in salt solution, and the resulting mixture under aseptic precautions was repeatedly injected directly into the liver which had been exposed by abdominal incision. The liver was selected on account of the excellent nourishment afforded by its abundant blood supply, and also on account of the ease with which new tissue could be differentiated.

Injections into the portal vein were tried, but abandoned, as death invariably resulted from thrombosis.

The results were controlled by injecting into animals of the same age embryonic cells which had been killed by boiling.

Positive results were obtained in more than one-fourth of the cases. The most frequent result was the formation of cartilaginous nodules of the size of a pea or smaller. As these nodules did not occur normally in animals of the same age and size, the writers argue that a differentiation had taken place.

Cartilaginous nodules were found in the periphery of the lung, evidently as the result of embolism. Some of these nodules showed calcification.

In order to test the effect of altered conditions in the liver, a sterile platinum wire was passed through the liver, and at its ends silver electrodes were sewed to the abdominal wall. Through this wire a current sufficient to warm it was passed for five minutes each day. As a result, proliferation was more marked. Adenoid tissue, epithelial-like cells, and pigmented cells were found in addition to cartilage.

Giant cells apparently due to irritation of foreign material were frequently seen. After a time the nodules began to be absorbed, and finally disappeared.

The writers arrived at the following conclusions:

Embryonic cells differentiate after implantation. The greatest differentiation occurs when very young cells are implanted.

This differentiation is a specific property of the cell, and is not dependent

on the demands of the entire adult organism. A functional stimulus is necessary for cell preservation and growth. A permanent existence for the new growth has not been demonstrated by these experiments.

They are not tumors, as they are not progressive; but while Cohnheim's theory is not confirmed, it is not disproved.

R. M. P.

## NEUROLOGICAL LITERATURE.

EDITH M. BRACE.

Literature for Review should be sent to Edith M. Brace,  
131 Park Avenue, Rochester, N. Y.

**Warrington, W. B.** Further Observations on the Structural Alterations Observed in Nerve Cells. *Jour. Physiol.*, 24: 464-478, 4 text figs., 1899.

Experiments were made to determine what changes in cells of the spinal cord would result from cutting off afferent, or both afferent and efferent, impulses. Cats were used. The animals were anesthetized and the posterior roots of the spinal nerves were cut on one side or on both sides, or both anterior and posterior roots were cut. In others the cord was transected, and in another series of experiments hemi-section of the cord with division of afferent roots on that side was made. Examination was made several days or a number of months after the operation. Sections were prepared by Held's method, i. e., fix in saturated solution of perchloride of mercury and pass through the grades of alcohol.

Cases where the afferent nerves were cut showed some alteration in the cells of the anterior horn, but not as much for the cervical as for the lumbosacral region. When both anterior and posterior roots were cut, all cells on the side of the lesion showed some changes, but usually less than when only the anterior root had been cut. The anterior group seems to be prominent for the ease with which its cells undergo chromatolysis.

The ultimate fate of the cell after the cutting of its axon seemed to depend not only on injury to the other parts of the neuron to which it belongs, but also on the extent of the injury to neurons in immediate functional relationship to it. A human subject, after enucleation of the eyeball, showed no difference in the oculo-motor nuclei of the two sides on examination after eighteen months. The gray matter of the cord showed changes below the level of lesion in cases of transection. Clark's column is especially affected; cells of the anterior region were only slightly affected. After both hemi-section and division of afferent roots, the cells in the anterior horn of that side showed few and in some cases no changes.

E. M. B.

**Goltz, Fr.** Beobachtungen an einen Affen mit verstümmeltem Grosshirn. *Archiv. f. Physiol.* 76: 411-426, Tf. 1, 1899.

The observations noted in this paper are especially interesting, from the fact that the ape upon which they were made lived eleven years after the operation on its brain, during which time it was the subject of experiments by Professor Goltz and his assistants.

The cortex was removed from the left frontal lobe, and about a year later, from the left parietal lobe. After each operation there was lameness in the



right side; the muscles of the right half of the head, neck, back, and tail were greatly weakened at first, but recovered their normal tonus. Chewing motions, controlled by the muscles of the right side of the cheek, were also affected. The animal afterwards learned to jump, but could not use its right hand and foot in trying to reach any particular point, as well as before. It reached for things with its left hand, and whenever only one hand was required preferred to use the left, but could use the right when both hands were required. When walking on the ground it used both feet, but often only the left hand. In climbing it made purposeless motions with the right hand. The right side retained its sensibility, and the animal could always locate the point of stimulation.

In disposition the ape was moody and malicious at first, and although it could be coaxed into good humor, it was liable to sudden fits of anger.

Hearing and taste were not affected, but the right visual field was affected by the operation. In general, the disturbances were similar to those noted in the dog for the same operation.

The ape was trained to use the right hand again for definite purposes. It learned to carry food to the mouth, and would shake hands with the right hand, but the fist opened with difficulty, and there was a marked preference for the use of the left hand. Motions of the right side were always imperfect.

Examination after death showed that the voluntary muscles of the right side had lost the cross striæ, while those of the left side were normal.

Goltz thinks there must be conducting tracts between the right side of the cerebrum and the muscles of the right side, and he also believes that changes connected with the increase of irritability occur in the unmutilated part of the brain and in the spinal cord.

E. M. B.

**Harrington, N. R., and Leaming, Edward.** The Reaction of *Amœba* to Lights of Different Colors. *Am. Jour. Physiol.*, 3: 9-18, pl. 1, 1899.

A number of experiments made upon *Amœba proteus* indicate that it possesses the same kind of sensitiveness to different intensities of light, and to light from different parts of the spectrum, that is shown by some of the lower plants.

The intensity of the light is a strong factor in retarding and accelerating motion, but the decisive factor is color.

White light stops the streaming motion of *amœba*, producing a tense, rigor-like condition which is followed, when the action of the light is continued for some time, by a spasmodic but usually futile attempt to form pseudopodia. This light rigor is considered identical with that demonstrated in *Nitella* and in bacteria. Red or green light causes the streaming to start, while violet light retards it, or will stop it completely in a few seconds. Yellow light starts motion and also reverses the direction of the pre-existing flow. The action of the different colors is relative and depends to some extent upon the order in which they are used. Violet light is inhibitive, but used after white light it produces flowing.

In some cases, when the water supply on the slide had become exhausted, the *amœba* ruptured and lost its nucleus; if this enucleated part was supplied with water, it gave the same reactions to light as the normal *amœba*.

The experiments gave no evidence in support of the theory that the circular, quiescent form of amœba represents full contraction. The writers believe that the distinction of entosarc and ectosarc is purely arbitrary, depending upon the distribution of granules in the protoplasm, and that it is not a fundamental difference.

Large amœbæ were used for the experiments and the motions were studied by means of images thrown upon a screen by a large electric projection lantern. Light filters of colored celloidin, used by Bierstadt in photographing colors, interposed between the amœba and the arc lamp produced the quick changes of color required. Although such light is not purely monochromatic, the same results were obtained as when monochromatic lights were used. Experiments of this sort are well suited for class demonstration.

E. M. B.

## NOTES ON RECENT MINERALOGICAL LITERATURE.

ALFRED J. MOSES AND LEA MCI. LUQUER.

Books and reprints for review should be sent to Alfred J. Moses, Columbia University, New York, N. Y.

**A. Arzruni and K. Thaddeff.** New Minerals from Chili. *Zeit. f. Kryst*, 31: 229, 1899. The late Prof. Arzruni left unfinished the description of several new Chilean minerals. This work has been completed by A. Dannenberg.

**ARZRUNITE.**—A brilliant bluish-green mineral occurring as minute crystals and druses in the hollows of a porous quartzose rock from Mina Buena Esperanza, Challocollo, Tarapaca, Chili.

The crystals are by measurement geometrically hexagonal prism, pyramid and base, but being strongly pleochroic at right angles to the vertical axis and biaxial in convergent light, they are described as orthorhombic with  $a : b : c = 0.5773 : 1 : 0.4163$ .

After deduction of numerous impurities the formula given is  $\text{PbSO}_4 \cdot \text{PbO} + 3(\text{Cu Cl}_2 \text{ H}_2 \text{ O}) + \text{Cu}(\text{OH})_2$ .

**STELZNERITE.**—Beautiful green transparent crystals of brilliant luster occurring upon a somewhat similar spongy mass of a brighter green color.

The crystals are orthorhombic, showing prism, pinacoids 010 and 001, pyramid and dome 011. The axial plane is parallel 001, the axes emerging almost perpendicular to the prism faces. Pleochroism, blue green to yellow green.  $a : b : c = 0.50368 : 1 : 0.70585$ .

Analyses yield formula  $\text{CuSO}_4 \cdot 2 \text{Cu}(\text{OH})_2$ . Mineral resembles brochantite. Locality, Remolinos, Vallinar, Chili.

**RAFAELITE.**—At Mina San Rafael, Sierra Gorda, Chili, there occur violet red needles 0.5 to 1 mm. long and 0.2 to 0.5 mm. broad, forming a drusy coating upon galenite, quartz and celestite. Schwarzenbergite is also found.

The crystals are monoclinic and show several domes terminating a vertical

zone of 110, 130, 010, 100. They are highly pleochroic, deep violet for vibrations parallel the length, and violet-red at right angles, and with convergent light both axes show in 100.

Axial ratio,  $a : b : c = 0.9034 : 1 : 1.2036$   $\beta = 117^\circ 13'$ .

A. J. M.

**Bourgeois Léon.**—Sur un chlorate basique de cuivre cristallisé. Bull. Soc. Min., **21**: 170, 1898.

Manner of making described and crystallographic and optical examination recorded.

Consists of very small, green, orthorhombic tablets, resembling gerhardtite and isomorphous with it. Insoluble in water, but easily soluble in acids.  $G. = 3.55$ . Analysis yields formula,  $4 \text{ CuO} \cdot \text{Cl}_2\text{O}_5 + 3 \text{ H}_2\text{O}$ , or  $(\text{Cl O}_3)_2 \text{ Cu} + 3 \text{ Cu (O H)}_2$ .

L. McL. L.

**V. Goldschmidt.** Ueber einen Krystallmodellir apparat. Zeit f. Kryst., **31**: 223, 1899.

Prefacing a description of an apparatus for cutting crystal models, Prof.

Goldschmidt points out that the use of models in the study of crystals may be divided into three stages:

1. Elementary instruction, in which models are more considered than actual crystals, the latter being employed only when they are simple and approximate the ideal or model form, such as octahedrons of magnetite, cubes of pyrite or dodecahedrons of garnet.

2. Higher instruction, in which the student commences to realize that in nature there are no perfect cubes or octahedrons, that crystals are usually small, partially formed, generally show combinations, and that one crystal in its formation often disturbs the formation of another. The student now measures, draws and calculates for himself, and gradually realizes the beauty and wonderful regularity of the crystals, and the models appear crude and incorrect and relatively valueless in comparison with projections.

3. A stage which might be called that of the investigator, in which models again are valued, which show directly what is being observed and what has been seen on similar material. Such models are not to be purchased.

The new apparatus is based upon the principle of the two-circle goniometer in which the position of any face is determined by the direction of a ray normal to the face. Two angles  $\varphi$  and  $\rho$ , which are recorded for all known forms of natural crystals, determine the *direction* of this ray with respect to a pole and a first meridian, and to locate the face it is only necessary to know the distance to the center.

A vertical circle ( $\varphi$  circle) and a horizontal circle ( $\rho$  circle) graduated to one degree are used. The axis of the  $\varphi$  circle carries a fragment of, say, gypsum or soapstone. A movable knife is attached to the horizontal circle at the angle given by  $\varphi$   $\rho$  and cuts away from the block layers parallel to the desired face until the desired central distance is attained. The largest faces are cut first.

The central distances, the author claims, will be in essentially the same ratio in the model and the crystal if the cutting is continued until to the eye the shapes of the faces are essentially those of the crystal.

These central distances determine the habit of the crystal and, as is known,

the habit varies little for crystals of the same substance formed under the same conditions. Prof. Goldschmidt proposes to seek by these models to determine the laws underlying the crystal habit.

A. J. M.

**Hartley, E. G. J.** On the Constitution of the Mineral Arsenates and Phosphates. Part I (Chalcophyllite). *Min Mag.*, **12**: 120, 1899.

7.04 = 100.43.

These numbers do not give a simple formula for the mineral, hence further analyses are necessary to determine true constitution.

L. McI. L.

A careful re-analysis of this mineral gives:  $\text{H}_2\text{O}$ , 28.26%;  $\text{CuO}$ , 45.93;  $\text{Al}_2\text{O}_3$ , 4.74;  $\text{As}_2\text{O}_5$ , 14.46;  $\text{SO}_3$ ,

## Received for the Journal Library.

(All publications received for this library are carefully filed and preserved for reference.)

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**Akromegaly.** With Report of a Case Presenting some Unusual Features; Height of Patient Eight Feet and Six Inches.

**American Postal Microscopical Club.** Twenty-fourth Annual Report, May 24, 1899, with an Appendix of Extracts from the Note Books.

**A Case of Diabetes Millitus Quickly Following Mumps.** H. F. Harris, M. D., Repr. Bost. Med. and Surg. Jour., May 18, 1899.

**A New Mineral Box and Rotating Stage-Holder for the Examination of Minerals under the Microscope.** Roy Hopping. Four pages, illustrated.

**An Improved Micro-Stereoscopic Camera.** John G. Baker.

**A Plea for the Protection of our Birds.** Professor Lawrence Bruner, University of Nebraska.

**Beihefte zum Botanischen Centralblatt.** Bd. VIII, H. 7.

**Boletin del Consejo Superior de Salubridad.** April, 1899.

**Brayton, A. W.** The Relations of the Surgeon and the Microscopist. *Ind. Med. Jour.* pp. 287-289, Feb. 1898.

**Buletino del Laboratorio ed Orto Botanico.**

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**Bulletin of the New York State Museum.** Vol. 5, No. 24, A Memorial of the Life and Entomologic Work of Joseph Albert Lintner, Ph. D., 1899. Vol. 6, No. 26, Collection, Preservation and Distribution of New York Insects, text figs., pp. 1-29, 1899; No. 27, Shade Tree Pests in New York State, pp. 40-60, 1899; No. 28, A Systematic Study of the Plants of North Elba.

**Deutsche Botanische Monatsschrift.** XVI. Heft 8, 1898.

**Fifteenth Annual Report of the Bureau of Animal Industry,** pp. 1-647, 1898.

**Flora of Lake Quinsigamond.** G. E. Stone, pp. 1-12.

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**Graham, St. Joseph B., M. D.** The Preservation of Culture Media.

\* Water lost at 100° C = 17.93.



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- Immunity and the Rationale of Vaccination.** O. P. Phillips, Ph. M.
- Journal of the Boston Society of Medical Sciences, October, 1899.** This number has three plates showing photographs of the parasites of tertian and æstivo-autumnal malarial fever.
- Journal of the Quekeet Microscopical Club.** Text figs. and 3 plates. April, 1899.
- Lackey, Wm. N., M. D.** Repr. Phila. Med. Jour., July 22, 1899. Outbreak of Chrysanthemum Rust, Byron D. Halsted.
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- Observations on Errant Frustules of Eunotia Major,** pp. 110-119, two plates, 1898. T. C. Palmer.
- On a Form of Degeneration of Striated Muscle Met with in the Uvula.** A. G. Hoen. Jour. Exp. Med. III, pp. 549-572, 9 plates. 1898.
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- Proceedings and Transactions of the Croydon Microscopical and Natural History Club,** February 15, 1898, to January 17, 1899.
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- The College, Past and Present.** M. J. Elrod.
- The Journal of the Franklin Institute.** Index of Publications, Vol. 146, Nos. 871-876. List of New Members, Jan. 1 to June 30, 1899.
- The Preservation of Culture Media.** S. B. Graham,
- The Propagation of Diseases by Means of Insects, with Special Consideration of the Common Domestic Types,** pp. 1-15. W. M. L. Coplin, M. D.
- Transactions of the Texas Academy of Science, 1897.** This number presents eleven papers on scientific and economic subjects. Transactions of 1898.
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- Veeder, M. A., M. D.** The Relative Importance of Flies and Water-Supply in Spreading Disease. Repr. from Med. Rec. pp. 1-8. Jan. 7, 1899.
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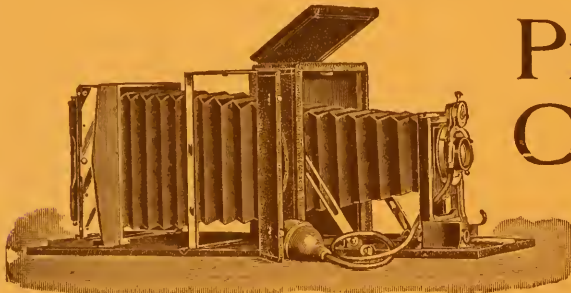
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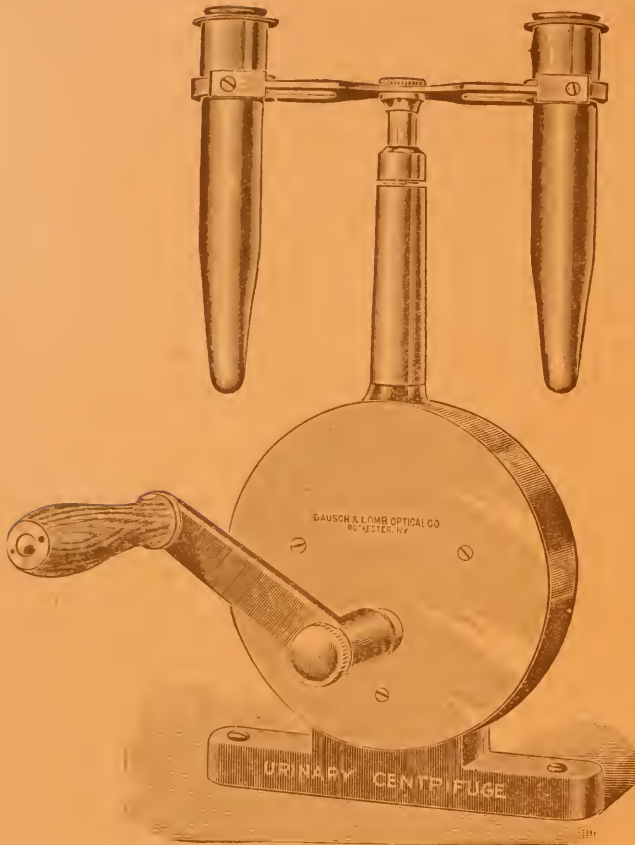


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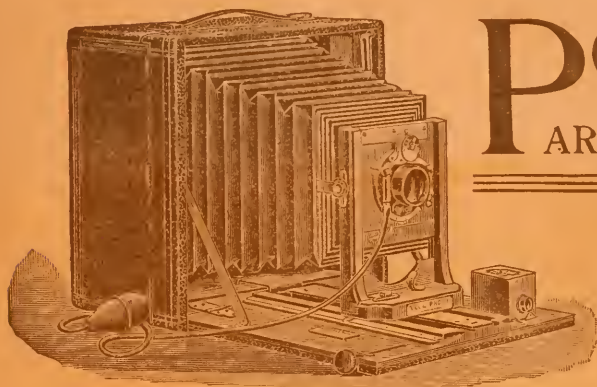
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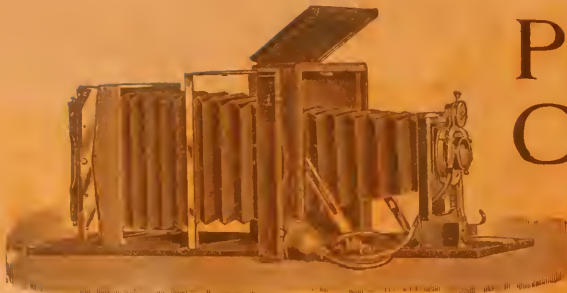
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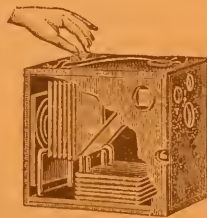
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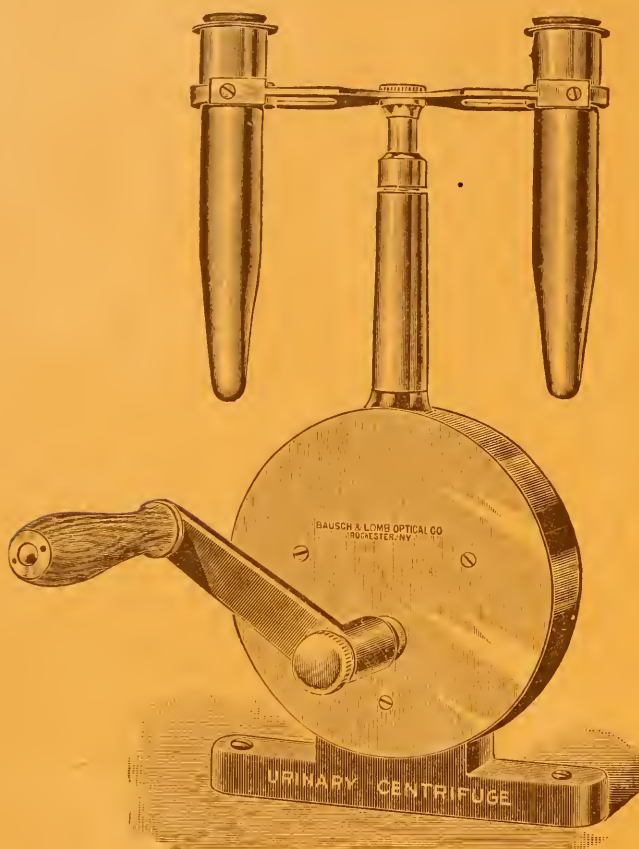


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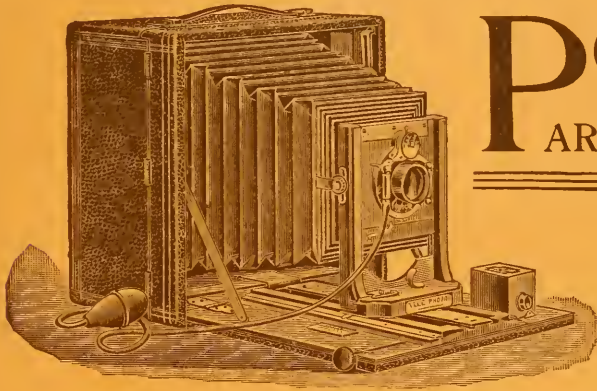
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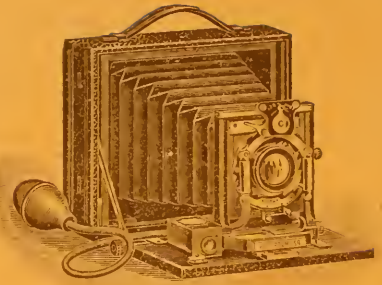
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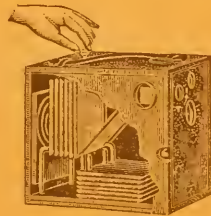
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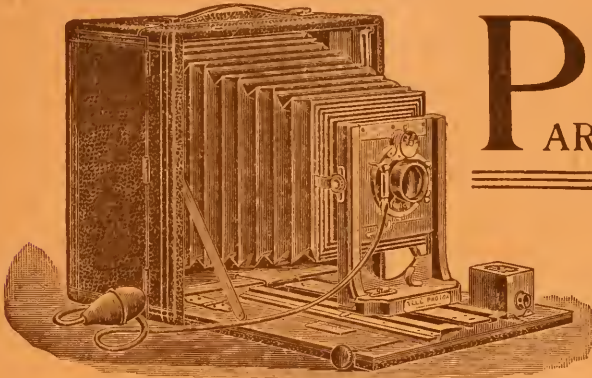


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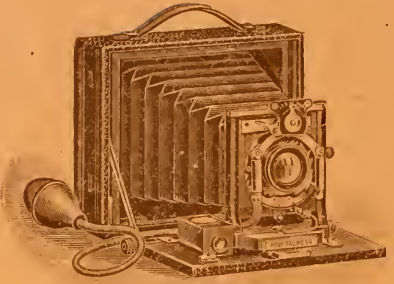
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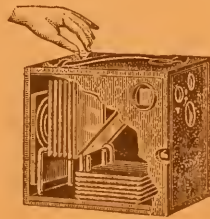
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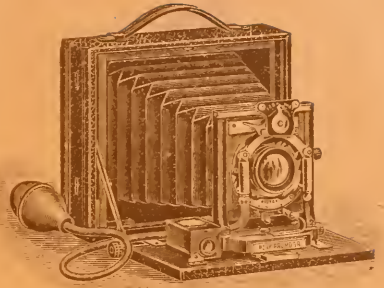
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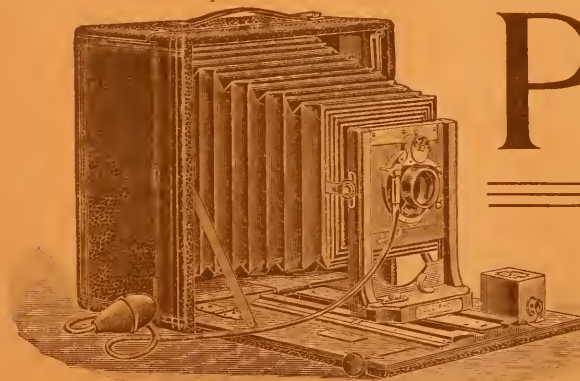
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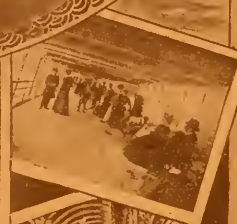
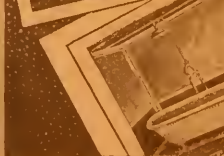
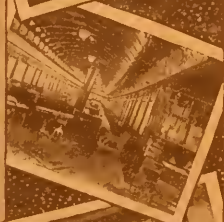
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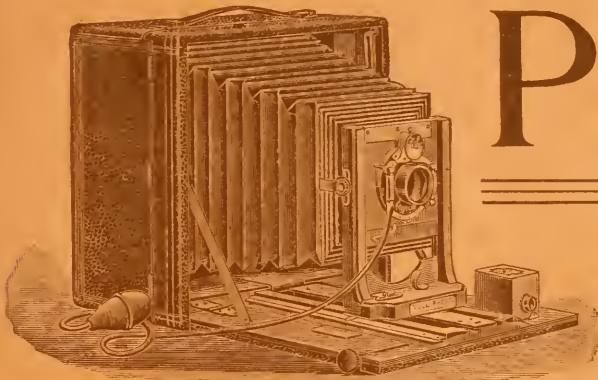
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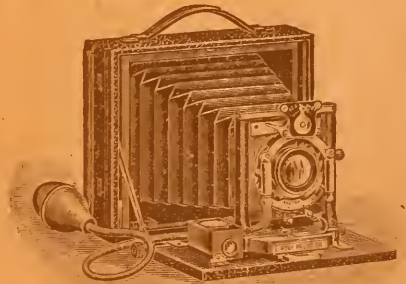
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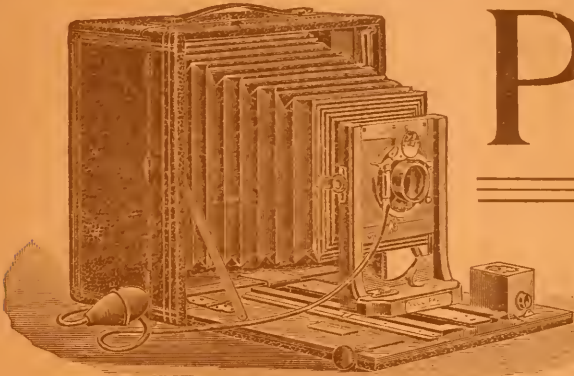
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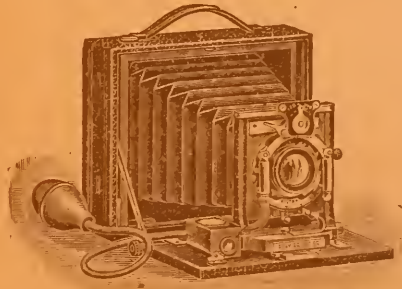
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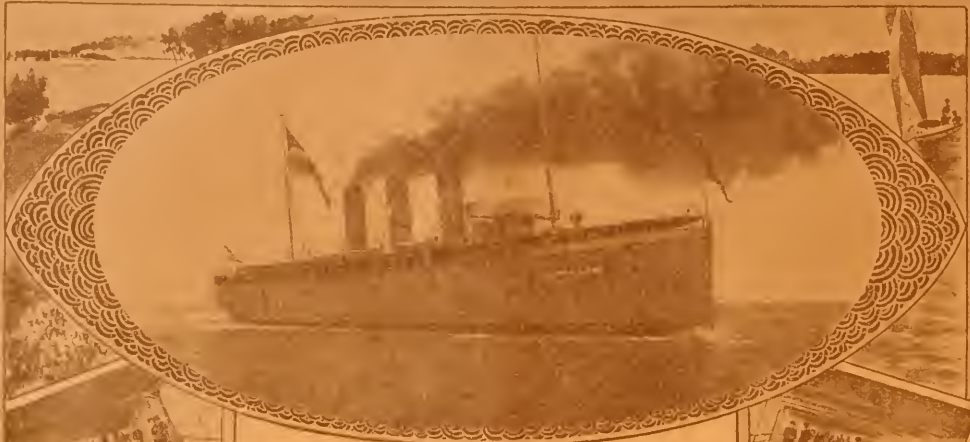
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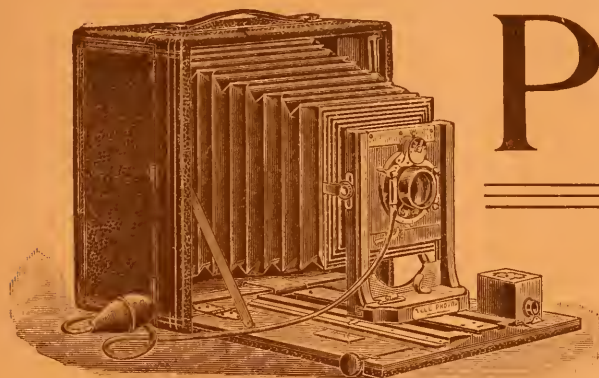
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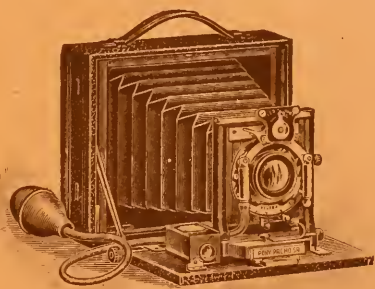
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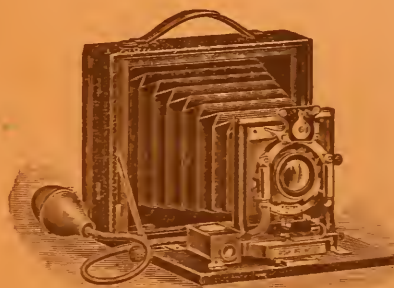
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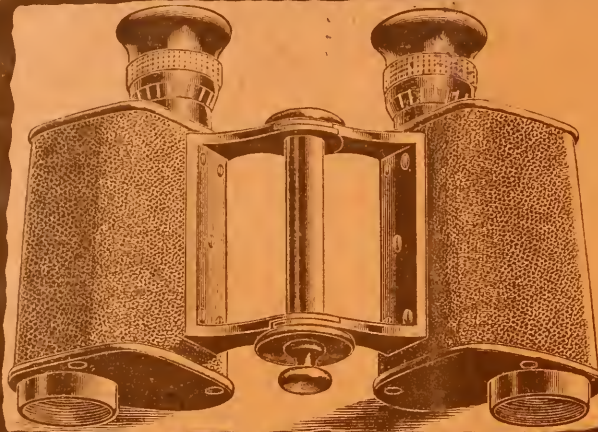
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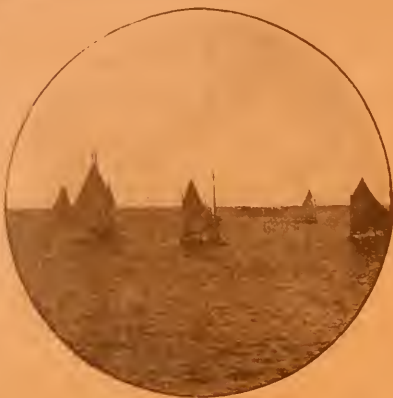
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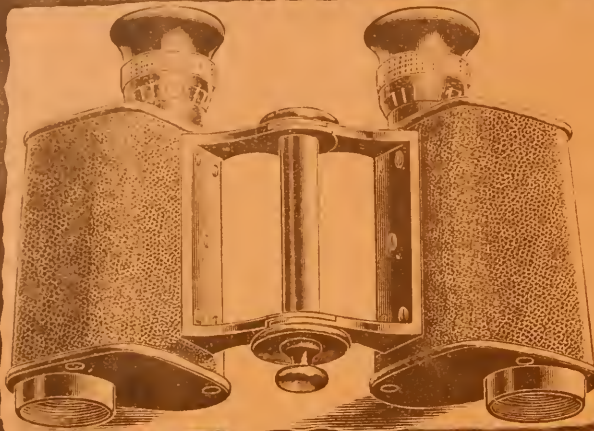
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